(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 April 2010 (01.04.2010)

PCT

(10) International Publication Number
WO 2010/036979 A2

(51) International Patent Classification:
C07K 14/555 (2006.01) *C07H 21/04* (2006.01)
C12N 15/63 (2006.01) *C12P 21/06* (2006.01)

(21) International Application Number:
PCT/US2009/058498

(22) International Filing Date:
25 September 2009 (25.09.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/100,116 25 September 2008 (25.09.2008) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

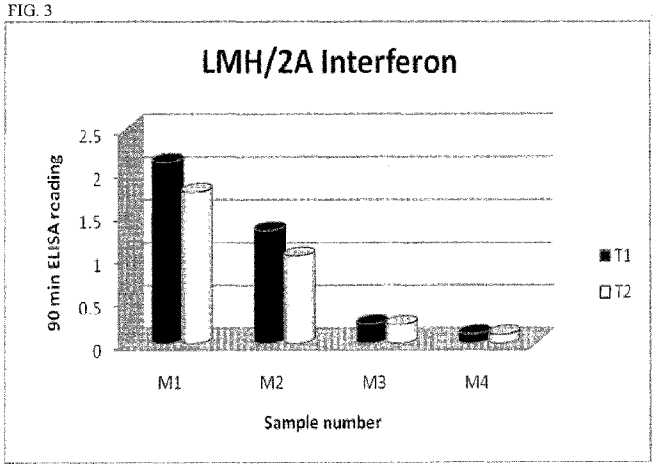
Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: NOVEL VECTORS FOR PRODUCTION OF INTERFERON

FIG. 3



(57) **Abstract**: Novel compositions for the production of interferons such as interferon-α 2a, interferon-α 2b, or interferon-β 1a (IFN-α 2a, IFN-α 2b, or IFN-β 1a) are provided. The compositions comprise components of vectors, such as a vector backbone, a promoter, and a gene of interest that encodes an interferon such as IFN-α 2a, IFN-α 2b, or IFN-β 1a, and the vectors comprising these components. In certain embodiments, these vectors are transposon-based vectors. Also provided are methods of making these compositions and methods of using these compositions for the production of an interferon such as IFN-α 2a, IFN-α 2b, or IFN-β 1a.

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NOVEL VECTORS FOR PRODUCTION OF INTERFERON

FIELD OF THE INVENTION

The present disclosure relates to compositions and methods for the production of interferon (IFN). In particular, the disclosure relates to transposon based vectors and their use in methods for the efficient expression of an interferon.

BACKGROUND OF THE INVENTION

Interferons are a family of proteins, produced by cells of the immune system, that provide protection against viruses, bacteria, tumors, and other foreign substances that may invade the body. There are three classes of interferons, and each class has different, but overlapping effects. Interferons attack a foreign substance, by slowing, blocking, or changing its growth or function.

Interferon alpha (IFN-α) proteins are closely related in structure, containing 165 or 166 amino acids, including four conserved cysteine residues which form two disulfide bridges. The IFN-α proteins include twelve different protein types (*e.g.*, 1, 2, etc.) which are encoded by about fourteen genes, and each of the protein types is further broken down into different subtypes (*e.g.*, a, b, etc.). To date, interferon alpha 2 (IFN-α2) has been used predominantly as a therapeutic. Pegylated and/or non-pegylated forms of interferon alpha 2a (IFN-α 2a (also sometimes referred to as INF-α 2a)) and interferon alpha 2b (IFN-α 2b (also sometimes referred to as INF-α 2b)) have received FDA approval for the treatment of hairy cell leukemia, malignant melanoma, follicular lymphoma, condylomata acuminate, AIDS-related Kaposi sarcoma, and chronic hepatitis B and C. IFN-α 2a, IFN-α 2b, and IFN-α 2c differ only by one or two amino acids from one another. Human leukocyte subtype IFN-αLe has been used in several European countries for adjuvant treatment of patients with stage IIb to stage III cutaneous melanoma after two initial cycles of dacarbazine (DTIC).

In addition, IFN-β proteins have been used as therapeutics. For example, IFN-β1a and IFN-β1b have been used to treat and control multiple sclerosis, by slowing progression and activity in relapsing-remitting multiple sclerosis and by reducing attacks in secondary progressive multiple sclerosis.

The manufacture of therapeutic interferons such as IFN-α 2a, IFN-α 2b, IFN-β1a, and IFN-β1b is an expensive process. Companies using recombinant techniques to manufacture these proteins are working at capacity and usually have a long waiting list to access their fermentation

facilities. What is needed, therefore, are new, efficient, and economical approaches to make interferons, such as IFN-α 2a, IFN-α 2b, IFN-β1a, and IFN-β1b, *in vitro* or *in vivo*.

SUMMARY

The present invention addresses these needs by providing novel compositions which can be used to transfect cells for production of an interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b. These compositions also can be used for the production of transgenic animals that can transmit the gene encoding an interferon to their offspring. These novel compositions include components of vectors such as a vector backbone (SEQ ID NOs:1-13), a novel promoter (SEQ ID NOs:14-15), and a gene of interest that encodes for an interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b. The present vectors further comprise an insulator element located between the transposon insertion sequences and the multicloning site on the vector. In one embodiment, the insulator element is selected from the group consisting of an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, and a matrix attachment region element. The expression vectors comprising these components are shown as SEQ ID NOs:17-28. In one embodiment these vectors are transposon-based vectors. The present invention also provides methods of making these compositions and methods of using these compositions for the production of an interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b. In one embodiment, the interferon is human (h)IFN-α 2a, hIFN-α 2b, hIFN-β1a, or hIFN-β1b.

It is to be understood that different cells may be transfected with one of the presently disclosed compositions, provided the cells contain protein synthetic biochemical pathways for the expression of the gene of interest. For example, both prokaryotic cells and eukaryotic cells may be transfected with one of the disclosed compositions. In certain embodiments, animal or plant cells are transfected. Animal cells include, for example, mammalian cells and avian cells. Animal cells that may be transfected include, but are not limited to, Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, human ARPT-19 (human pigmented retinal epithelial) cells, LMH cells, LMH2a cells, tubular gland cells, or hybridomas.

In one embodiment, avian cells are transfected with one of the disclosed compositions. In a specific embodiment, avian hepatocytes, hepatocyte-related cells, or tubular gland cells are transfected. In certain embodiments, chicken cells are transfected with one of the disclosed

compositions. In one embodiment, chicken tubular gland cells, chicken embryonic fibroblasts, chicken LMH2A cells, or chicken LMH cells are transfected with one of the disclosed compositions. Chicken LMH and LMH2A cells are chicken hepatoma cell lines; LMH2A cells have been transformed to express estrogen receptors on their cell surface.

In other embodiments, mammalian cells are transfected with one of the disclosed compositions. In one embodiment, Chinese hamster ovary (CHO) cells, ARPT-19 cells, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, or hybridomas are transfected for IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b production. In a specific embodiment, CHO-K1 cells or ARPT-19 cells are transfected with one of the disclosed compositions.

The present disclosure provides compositions and methods for efficient production of interferons such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b, particularly human interferons such as hIFN-α 2a, hIFN-α 2b, hIFN-β1a, or hIFN-β1b. These methods enable production of large quantities of interferons such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b. In some embodiments, when the present compositions are used for *in vitro* expression, the interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b is produced at a level of between about 25 g protein/month and about 4 kg protein/month.

These vectors also may be used *in vivo* to transfect germline cells in animals such as birds which can be bred and which then pass an IFN transgene through several generations. These vectors also may be used for the production of an IFN *in vivo*, for example, for deposition in an egg.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the structure of two different hybrid promoters. Figure 1A is a schematic of the Version 1 CMV/Oval promoter 1 (ChOvp/CMVenh/CMVp; SEQ ID NO:14). Figure 1B is a schematic of the Version 2 CMV/Oval promoter (SEQ ID NO:15; ChSDRE/CMVenh/ChNRE/CMVp).

Figure 2A is a schematic showing the #188 vector (SEQ ID NO:17) used for expression of hIFN-α 2b. Figure 2B is a schematic showing the #206 vector (SEQ ID NO:18) used for expression of hIFN-α 2b. Figure 2C is a schematic showing the #207 vector (SEQ ID NO:19) used for expression of hIFN-α 2b. Figure 2D is a schematic showing the general structure of the resulting hIFN-α 2b transcript from the expression vectors. The signal sequence is translated, but is cleaved in the endoplasmic reticulum and is not part of the resulting 3xFlag hIFN-α 2b protein.

Figure 3 is a graph showing the results of an enzyme linked immunosorbent assay (ELISA) demonstrating the efficient expression of 3xFlag hIFN-α 2b in LMH2A cells using the

#188 expression vector (SEQ ID NO:17) described herein. T1 (the left bar of each pair) and T2 (the right bar of each pair) reflect duplicate flasks. Control flasks also were run, but exhibited readings that were too low to detect (data not shown). M1 is 2 days post-transfection; M2 is 5 days post-transfection; M3 is 7 days post-transfection; and M4 is 9 days post-transfection. The Y axis is a measurement of absorbance at 405 nm. These cells were not under selection pressure. The #206 vector (SEQ ID NO:18), #207 vector (SEQ ID NO:19), #261 vector (SEQ ID NO:20), #262 vector (SEQ ID NO:21), #248 vector (SEQ ID NO:22), #309 vector (SEQ ID NO:23), #310 vector (SEQ ID NO:24), #311 vector (SEQ ID NO:25), and #295 vector (SEQ ID NO:28) also efficiently expressed 3xFlag hIFN-α 2b (see Table 4 below).

Figure 4 is a graph showing the results of a sandwich enzyme linked immunosorbent assay (ELISA) demonstrating the efficient expression of mature hIFN-α 2b in LMH2A cells using the #248 expression vector (SEQ ID NO:22) described herein. T1, T2, and T3 (left panel) are three separate flasks of LMH2A cells transfected with the #206 expression vector (3xFlag hIFN-α2b) (SEQ ID NO:18), and T4, T5, and T6 (right bar panel) are three separate flasks of LMH2A cells transfected with the #248 expression vector (native hIFN-α2b). Control flasks also were run, but exhibited readings that were too low to detect (data not shown). M1 (left bar of each group) is 2 days post-transfection; M2 (middle bar of each group) is 6 days post-transfection; and M3 (right bar of each group) is 9 days post-transfection.

Figure 5 is a graph showing the results of a sandwich enzyme linked immunosorbent assay (ELISA) demonstrating the efficient expression of 3xFlag hIFN-α 2b and mature hIFN-α 2b in LMH and LMH2A cells using the #206 expression vector (SEQ ID NO:18) or the #248 expression vector (SEQ ID NO:22) described herein. T1, T2, and T3 (left panel) and T13, T14, and T15 (left center panel) are three separate flasks of LMH cells or LMH2A cells, respectively, transfected with the #206 expression vector (3xFlag hIFN-α2b). T10, T11, and T12 (right center panel) and T22, T23, and T24 (right panel) are three separate flasks of LMH cells or LMH2A cells, respectively, transfected with the #248 expression vector (native hIFN-α2b). Control flasks also were run, but exhibited readings that were too low to detect (data not shown). M1 (left bar of each group) is 3 days post-transfection; M2 (middle bar of each group) is 7 days post-transfection; and M3 (right bar of each group) is 10 days post-transfection.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

The present invention provides novel vectors and vector components for use in transfecting cells for production of interferons such as hIFN-α 2a, hIFN-α 2b, hIFN-β1a, or hIFN-β1b *in vitro* or *in vivo*. The present invention also provides methods to make these vector

components, methods to make the vectors themselves, and methods for using these vectors to transfect cells such that the transfected cells produce the interferon. The inteferon may be any interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, hIFN-β1b, hIFN-α Le, hIFN-g, or others known to one of skill in the art. In some embodiments, the interferon is a human interferon such as hIFN-α 2a, hIFN-α 2b, hIFN-β1a, or hIFN-β1b. Any cell with protein synthetic capacity may be used for this purpose. Animal cells are the preferred cells, particularly mammalian cells and avian cells. Animal cells that may be transfected include, but are not limited to, Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, human ARPT-19 (human pigmented retinal epithelial) cells, LMH cells, LMH2a cells, tubular gland cells, or hybridomas. Avian cells include, but are not limited to, LMH, LMH2a cells, chicken embryonic fibroblasts, and tubular gland cells.

As used herein, the terms "interferon," "IFN," "interferon α 2," "IFN-α 2a," "IFN-α 2b," "IFN-β 1a," and "IFN-β1b" refer to an interferon protein that is encoded by a gene that is either a naturally occurring or a codon-optimized gene. As used herein, the term "codon-optimized" means that the DNA sequence has been changed such that where several different codons code for the same amino acid residue, the sequence selected for the gene is the one that is most often utilized by the cell in which the gene is being expressed. For example, in some embodiments, the interferon gene is expressed in LMH or LMH2A cells and includes codon sequences that are preferred in that cell type. In one embodiment, the interferon gene is an hIFN-α 2a gene, an hIFN-α 2b gene, an hIFN-β1a gene, or an hIFN-β1b gene. In one embodiment, the gene is shown in nucleotides 6714-7211 of SEQ ID NO:17. In other embodiments, the interferon is an interferon other than IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b, the sequence of which may be found by one of skill in the art in sequence databases such as GenBank.

In one embodiment, the vectors of the present invention contain a gene encoding an interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b for the production of such protein by transfected cells *in vitro*. In other embodiments, the interferon such as IFN-α 2a, IFN-α 2b, IFN-β1a, or IFN-β1b for the production of such protein by transfected cells *in vivo*.

A. Vectors & Vector Components

The following paragraphs describe the novel vector components and vectors employed in the present invention.

1. *Backbone Vectors*

The backbone vectors provide the vector components minus the gene of interest (GOI) that codes for the interferon. In one embodiment, transposon-based vectors are used as described further under sections 1.a. through 1.m.

a. *Transposon-Based Vector Tn-MCS #5001 (p5001) (SEQ ID NO:1)*

Linear sequences were amplified using plasmid DNA from pBluescriptII sk(-) (Stratagene, La Jolla, CA), pGWIZ (Gene Therapy Systems, San Diego, CA), pNK2859 (Dr. Nancy Kleckner, Department of Biochemistry and Molecular Biology, Harvard University), and synthetic linear DNA constructed from specifically designed DNA Oligonucleotides (Integrated DNA Technologies, Coralville, IA). PCR was set up using the above referenced DNA as template, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using Zymo Research's Clean Gel Recovery Kit (Orange, CA). The resulting products were cloned into the Invitrogen's PCR Blunt II Topo plasmid (Carlsbad, CA) according to the manufacturer's protocol.

After sequence verification, subsequent clones were selected and digested from the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) with corresponding enzymes (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The linear pieces were ligated together using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated products were transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread to LB (Luria-Bertani) agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was used as a sequencing template to verify that the pieces were ligated together accurately to form the desired vector sequence. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that consisted of the desired sequence, the DNA was isolated for use in cloning in specific genes of interest.

b. *Preparation of Transposon-Based Vector TnX-MCS #5005 (p5005)*

This vector (SEQ ID NO:2) is a modification of p5001 (SEQ ID NO:1) described above in section 1.a. The MCS extension was designed to add unique restriction sites to the multiple cloning site of the pTn-MCS vector (SEQ ID NO:1), creating pTnX-MCS (SEQ ID NO:2), in order to increase the ligation efficiency of constructed cassettes into the backbone vector. The first step was to create a list of all non-cutting enzymes for the current pTn-MCS DNA sequence (SEQ ID NO:1). A linear sequence was designed using the list of enzymes and compressing the restriction site sequences together. Necessary restriction site sequences for XhoI and PspOMI (New England Biolabs, Beverly, MA) were then added to each end of this sequence for use in splicing this MCS extension into the pTn-MCS backbone (SEQ ID NO:1). The resulting sequence of 108 bases is SEQ ID NO:16 shown in the Appendix. A subset of these bases within this 108 base pair sequence corresponds to bases 4917-5012 in SEQ ID NO:4 (discussed below).

For construction, the sequence was split at the NarI restriction site and divided into two sections. Both 5' forward and 3' reverse oligonucleotides (Integrated DNA Technologies, San Diego, CA) were synthesized for each of the two sections. The 5' and 3' oligonucleotides for each section were annealed together, and the resulting synthetic DNA sections were digested with NarI then subsequently ligated together to form the 108 bp MCS extension (SEQ ID NO:16). PCR was set up on the ligation, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The resulting product was cloned into the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol.

After sequence verification of the MCS extension sequence (SEQ ID NO:16), a clone was selected and digested from the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) with XhoI and PspoMI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The pTn-MCS vector (SEQ ID NO:1) also was digested with XhoI and PspOMI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol, purified as described above, and the two pieces were ligated together using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at

37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 mls of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the multiple cloning site extension, the DNA was isolated and used for cloning specific genes of interest.

c. *Preparation of Transposon-Based Vector TnHS4FBV #5006 (p5006)*

This vector (SEQ ID NO:3) is a modification of p5005 (SEQ ID NO:2) described above in section 1.b. The modification includes insertion of the HS4 βeta globin insulator element on both the 5' and 3' ends of the multiple cloning site. The 1241 bp HS4 element was isolated from chicken genomic DNA and amplified through polymerase chain reaction (PCR) using conditions known to one skilled in the art. The PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size of the HS4 βeta globin insulator element were excised from the agarose gel and purified using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified HS4 DNA was digested with restriction enzymes NotI, XhoI, PspOMI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The digested DNA was then purified using a Zymo DNA Clean and Concentrator kit (Orange, CA). To insert the 5' HS4 element into the MCS of the p5005 vector (SEQ ID NO:2), HS4 DNA and vector p5005 (SEQ ID NO:2) were digested with NotI and XhoI restriction enzymes, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. To insert the 3' HS4 element into the MCS of the p5005 vector (SEQ ID NO:2), HS4 and vector p5005 DNA (SEQ ID NO:2) were digested with PspOMI and MluI, purified, and ligated as described above. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting

colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 mls of LB/amp broth and plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as sequencing template to verify that any changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both HS4 elements, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

d. *Preparation of Transposon-Based Vector pTn10 HS4FBV #5012*

This vector (SEQ ID NO:4) is a modification of p5006 (SEQ ID NO:3) described above under section 1.c. The modification includes a base pair substitution in the transposase gene at base pair 1998 of p5006. The corrected transposase gene was amplified by PCR from template DNA, using PCR conditions known to one skilled in the art. PCR product of the corrected transposase was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified transposase DNA was digested with restriction enzymes NruI and StuI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction digests using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the corrected transposase sequence into the MCS of the p5006 vector (SEQ ID NO:3), the transposase DNA and the p5006 vector (SEQ ID NO:3) were digested with NruI and StuI, purified as described above, and ligated using a Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC

(GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C before spreading onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth. The plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the corrected transposase sequence, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest was grown in 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

e. *Preparation of Transposon-Based Vector pTn-10 MARFBV #5018*

This vector (SEQ ID NO:5) is a modification of p5012 (SEQ ID NO:4) described above under section 1.d. The modification includes insertion of the chicken 5' Matrix Attachment Region (MAR) on both the 5' and 3' ends of the multiple cloning site. To accomplish this, the 1.7 kb MAR element was isolated from chicken genomic DNA and amplified by PCR. PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified MAR DNA was digested with restriction enzymes NotI, XhoI, PspOMI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from agarose using a Zymo DNA Clean and Concentrator kit (Zymo Research, Orange CA). To insert the 5' MAR element into the MCS of p5012, the purified MAR DNA and p5012 were digested with Not I and Xho I, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. To insert the

3' MAR element into the MCS of p5012, the purified MAR DNA and p5012 were digested with PspOMI and MluI, purified, and ligated as described above. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C and then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth, and plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both MAR elements, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

f. *Preparation of Transposon-Based Vector TnLysRep #5020*

The vector (SEQ ID NO:6) included the chicken lysozyme replicator (LysRep or LR2) insulator elements to prevent gene silencing. Each LysRep element was ligated 3' to the insertion sequences (IS) of the vector. To accomplish this ligation, a 930 bp fragment of the chicken LysRep element (GenBank # NW 060235) was amplified using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified LysRep DNA was sequentially digested with restriction enzymes Not I and Xho I (5'end) and Mlu I and Apa I (3'end) (New England Biolabs, Beverly, MA) according to the

manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the LysRep elements between the IS left and the MCS in pTnX-MCS (SEQ ID NO:2), the purified LysRep DNA and pTnX-MCS were digested with Not I and Xho I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB media (broth or agar) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C, and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the 5' LysRep DNA, the vector was digested with Mlu I and Apa I as was the purified LysRep DNA. The same procedures described above were used to ligate the LysRep DNA into the backbone and verify that it was correct. Once a clone was identified that contained both LysRep elements, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

g. *Preparation of Transposon-Based Vector TnPuro #5019 (p5019)*

This vector (SEQ ID NO:7) is a modification of p5012 (SEQ ID NO:4) described above in section 1.d. The modification includes insertion of the puromycin gene in the multiple cloning site adjacent to one of the HS4 insulator elements. To accomplish this ligation, the 602 bp puromycin gene was isolated from the vector pMOD Puro (Invivogen, Inc.) using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1%

agarose gel, stained with ethidium bromide, and visualized on a U.V. transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified Puro DNA was digested with restriction enzyme Kas I (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the Puro gene into the MCS of p5012, the purified Puro DNA and p5012 were digested with Kas I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both Puro gene, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

h. *Preparation of Transposon-Based Vector pTn-10 PuroMAR #5021 (p5021)*

This vector (SEQ ID NO:8) is a modification of p5018 (SEQ ID NO:5) described above in section 1.e. The modification includes insertion of the puromycin (puro) gene into the multiple cloning site adjacent to one of the MAR insulator elements. To accomplish this, the 602 bp puromycin gene was amplified by PCR from the vector pMOD Puro (Invitrogen Life

Technologies, Carlsbad, CA). Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified DNA from the puromycin gene was digested with the restriction enzymes BsiWI and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from agarose using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the puro gene into the MCS of p5018, puro and p5018 were digested with BsiWI and MluI, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. The plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was used as a sequencing template to verify that the changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the puro gene, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid of interest was grown in 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

i. *Preparation of Transposon-Based Vector TnGenMAR #5022 (p5022)*

This vector (SEQ ID NO:9) is a modification of p5021 (SEQ ID NO:8) described above under section 1.h. The modification includes insertion of the gentamycin gene in the multiple cloning site adjacent to one of the MAR insulator elements. To accomplish this ligation, the 1251

bp gentamycin gene was isolated from the vector pS65T-C1(ClonTech Laboratories, using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified gentamycin DNA was digested with restriction enzyme BsiW I and Mlu I (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the gentamycin gene into the MCS of p5018, the purified gentamycin DNA and p5018 were digested with BsiW I and Mlu I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C, and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both Puro gene, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

j. *Preparation of Low Expression CMV Tn PuroMAR Flanked Backbone #5024 (p5024)*

This vector (SEQ ID NO:10) is a modification of p5018 (SEQ ID NO:5), which includes the deletion of the CMV Enhancer region of the transposase cassette. The CMV enhancer was removed from p5018 by digesting the backbone with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size of the backbone without the enhancer region was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Backbone DNA from above was re-circularized using an Epicentre Fast Ligase Kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. The ligation was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5ml of LB/amp broth. Plasmid DNA was harvested using Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as a sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified containing the replacement promoter fragment, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

k. *Preparation of Low Expression CMV Tn PuroMAR Flanked Backbone #5025 (p5025)*

This vector (SEQ ID NO:11) is a modification of p5021 (SEQ ID NO:8), which includes the deletion of the CMV Enhancer of on the transposase cassette. The CMV enhancer was removed from p5021 by digesting the backbone with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size of the backbone without the enhancer region was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Backbone DNA from above was re-circularized using an Epicentre Fast Ligase Kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. The ligation was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB (Luria-Bertani) agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5 ml of LB/amp broth. Plasmid DNA was harvested using Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as a sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified containing the replacement promoter fragment, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

1. *Preparation of Low Expression SV40 promoter Tn PuroMAR Flanked Backbone #5026 (p5026)*

This vector (SEQ ID NO:12) is a modification of p5018 (SEQ ID NO:5), which includes the replacement of the CMV Enhanced promoter of the transposase cassette, with the SV40 promoter from pS65T-C1 (Clontech, Mountainview, CA). The CMV enhanced promoter was removed from p5018 by digesting the backbone with MscI and AfeI restriction enzymes. (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The SV40 promoter fragment was amplified to add the 5' and 3' cut sites, MscI and AscI, respectively. The PCR product was then cloned into pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA). Sequence verified DNA was then digested out of the pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA), with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified digestion product was ligated into the excised backbone DNA using Epicentre's Fast Ligase Kit (Madison, WI) according to the manufacturer's protocol. The ligation product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37° C before then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5 ml of LB/amp broth. The plasmid DNA was harvested using a Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once

a clone was identified that contained the replacement promoter fragment, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

m. *Preparation of Low Expression SV40 promoter Tn PuroMAR Flanked Backbone #5027 (p5027)*

This vector (SEQ ID NO:13) is a modification of p5021 (SEQ ID NO:8), which includes the replacement of the CMV Enhanced promoter of the transposase cassette, with the SV40 promoter from pS65T-C1 (Clontech, Mountainview, CA). The CMV enhanced promoter was removed from p5021 by digesting the backbone with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The SV40 promoter fragment was amplified to add the 5' and 3' cut sites, MscI and AscI, respectively. The PCR product was then cloned into pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA). Sequence verified DNA was then digested out of the pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA), with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified digestion product was ligated into the excised backbone DNA using Epicentre's Fast Ligase Kit (Madison, WI) according to the manufacturer's protocol. The ligation product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 µl of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C before being spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for

overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5 ml of LB/amp broth. The plasmid DNA was harvested using a Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the replacement promoter fragment, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

2. *Promoters*

A second embodiment of this invention are hybrid promoters that consist of elements from the constitutive CMV promoter and the estrogen inducible ovalbumin promoter. The goal of designing these promoters was to couple the high rate of expression associated with the CMV promoter with the estrogen inducible function of the ovalbumin promoter. To accomplish this goal, two hybrid promoters, designated versions 1 and 2 (SEQ ID NOs:14 and 15, respectively)(Figure 1), were designed, built, and tested in cell culture using a gene other than an interferon gene. Both versions 1 and 2 provided high rates of expression.

a. Version 1 CMV/Oval promoter 1 = ChOvp/CMVenh/CMVp

Hybrid promoter version 1 (SEQ ID NO:14) was constructed by ligating the chicken ovalbumin promoter regulatory elements to the 5' end of the CMV enhancer and promoter. A schematic is shown in Figure 1A.

Hybrid promoter version 1 was made by PCR amplifying nucleotides 1090 to 1929 of the ovalbumin promoter (GenBank # J00895) from the chicken genome and cloning this DNA fragment into the pTopo vector (Invitrogen, Carlsbad, CA). Likewise, nucleotides 245-918 of the CMV promoter and enhancer were removed from the pgWiz vector (ClonTech, Mountain View, CA) and cloned into the pTopo vector. By cloning each fragment into the multiple cloning site of

the pTopo vector, an array of restriction enzyme sites were available on each end of the DNA fragments which greatly facilitated cloning without PCR amplification. Each fragment was sequenced to verify it was the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin promoter fragment was digested with Xho I and EcoR I, and the product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the CMV promoter was treated in the same manner to open up the plasmid 5' to the CMV promoter; these restriction enzymes also allowed directional cloning of the ovalbumin promoter fragment upstream of CMV.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

b. Version 2 CMV/Oval promoter = ChSDRE/CMVenh/ChNRE/CMVp

Hybrid promoter version 2 (SEQ ID NO:15) consisted of the steroid dependent response element (SDRE) ligated 5' to the CMV enhancer (enh) and the CMV enhancer and promoter separated by the chicken ovalbumin negative response element (NRE).

A schematic is shown in Figure 1B. Hybrid promoter version 2 was made by PCR amplifying the steroid dependent response element (SDRE), nucleotides 1100 to 1389, and nucleotides 1640 to 1909 of the negative response element (NRE) of the ovalbumin promoter (GenBank # J00895) from the chicken genome and cloning each DNA fragment into the pTopo vector. Likewise, nucleotides 245-843 of the CMV enhancer and nucleotides 844-915 of the CMV promoter were removed from the pgWiz vector and each cloned into the pTopo vector. By cloning each piece into the multiple cloning site of the pTopo vector, an array of restriction enzyme sites were available on each end of the DNA fragments which greatly facilitated cloning without PCR amplification.

Each fragment was sequenced to verify it was the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin SDRE fragment was digested with Xho I and EcoR I to remove the SDRE, and the product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the CMV

enhancer was treated in the same manner to open up the plasmid 5' to the CMV enhancer; these restriction enzymes also allowed directional cloning of the ovalbumin SDRE fragment upstream of CMV. The ovalbumin NRE was removed from pTopo using NgoM IV and Kpn I; the same restriction enzymes were used to digest the pTopo clone containing the CMV promoter to allow directional cloning of the NRE.

The DNA fragments were purified as described above. The new pTopo vectors containing the ovalbumin SDRE/CMV enhancer and the NRE/CMV promoter were sequence verified for the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin SDRE/CMV enhancer fragment was digested with Xho I and NgoM IV to remove the SDRE/CMV Enhancer, and the product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the NRE/CMVpromoter was treated in the same manner to open up the plasmid 5' to the CMV enhancer. These restriction enzymes also allowed directional cloning of the ovalbumin SDRE fragment upstream of CMV. The resulting promoter hybrid was sequence verified to insure that it was correct.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

3. *Transposases and Insertion Sequences and Insulator Elements*

In a further embodiment of the present invention, the transposase found in the transposase-based vector is an altered target site (ATS) transposase and the insertion sequences are those recognized by the ATS transposase. However, the transposase located in the transposase-based vectors is not limited to a modified ATS transposase and can be derived from any transposase. Transposases known in the prior art include those found in AC7, Tn5SEQ1, Tn916, Tn951, Tn1721, Tn 2410, Tn1681, Tn1, Tn2, Tn3, Tn4, Tn5, Tn6, Tn9, Tn10, Tn30, Tn101, Tn903, Tn501, Tn1000 (γδ), Tn1681, Tn2901, AC transposons, Mp transposons, Spm transposons, En transposons, Dotted transposons, Mu transposons, Ds transposons, dSpm transposons and I transposons. According to the present invention, these transposases and their regulatory sequences are modified for improved functioning as follows: a) the addition one or more modified Kozak sequences comprising any one of SEQ ID NOs:31 to 40 at the 3' end of the

promoter operably-linked to the transposase; b) a change of the codons for the first several amino acids of the transposase, wherein the third base of each codon was changed to an A or a T without changing the corresponding amino acid; c) the addition of one or more stop codons to enhance the termination of transposase synthesis; and/or, d) the addition of an effective polyA sequence operably-linked to the transposase to further enhance expression of the transposase gene.

Although not wanting to be bound by the following statement, it is believed that the modifications of the first several N-terminal codons of the transposase gene increase transcription of the transposase gene, in part, by increasing strand dissociation. It is preferable that between approximately 1 and 20, more preferably 3 and 15, and most preferably between 4 and 12 of the first N-terminal codons of the transposase are modified such that the third base of each codon is changed to an A or a T without changing the encoded amino acid. In one embodiment, the first ten N-terminal codons of the transposase gene are modified in this manner. It is also preferred that the transposase contain mutations that make it less specific for preferred insertion sites and thus increases the rate of transgene insertion as discussed in U.S. Patent No. 5,719,055.

In some embodiments, the transposon-based vectors are optimized for expression in a particular host by changing the methylation patterns of the vector DNA. For example, prokaryotic methylation may be reduced by using a methylation deficient organism for production of the transposon-based vector. The transposon-based vectors may also be methylated to resemble eukaryotic DNA for expression in a eukaryotic host.

Transposases and insertion sequences from other analogous eukaryotic transposon-based vectors that can also be modified and used are, for example, the Drosophila P element derived vectors disclosed in U.S. Patent No. 6,291,243; the Drosophila mariner element described in Sherman et al. (1998); or the sleeping beauty transposon. See also Hackett et al. (1999); D. Lampe et al., 1999. Proc. Natl. Acad. Sci. USA, 96:11428-11433; S. Fischer et al., 2001. Proc. Natl. Acad. Sci. USA, 98:6759-6764; L. Zagoraiou et al., 2001. Proc. Natl. Acad. Sci. USA, 98:11474-11478; and D. Berg et al. (Eds.), Mobile DNA, Amer. Soc. Microbiol. (Washington, D.C., 1989). However, it should be noted that bacterial transposon-based elements are preferred, as there is less likelihood that a eukaryotic transposase in the recipient species will recognize prokaryotic insertion sequences bracketing the transgene.

Many transposases recognize different insertion sequences, and therefore, it is to be understood that a transposase-based vector will contain insertion sequences recognized by the particular transposase also found in the transposase-based vector. In a preferred embodiment of the invention, the insertion sequences have been shortened to about 70 base pairs in length as

compared to those found in wild-type transposons that typically contain insertion sequences of well over 100 base pairs.

While the examples provided below incorporate a "cut and insert" Tn10 based vector that is destroyed following the insertion event, the present invention also encompasses the use of a "rolling replication" type transposon-based vector. Use of a rolling replication type transposon allows multiple copies of the transposon/transgene to be made from a single transgene construct and the copies inserted. This type of transposon-based system thereby provides for insertion of multiple copies of a transgene into a single genome. A rolling replication type transposon-based vector may be preferred when the promoter operably-linked to gene of interest is endogenous to the host cell and present in a high copy number or highly expressed. However, use of a rolling replication system may require tight control to limit the insertion events to non-lethal levels. Tn1, Tn2, Tn3, Tn4, Tn5, Tn9, Tn21, Tn501, Tn551, Tn951, Tn1721, Tn2410 and Tn2603 are examples of a rolling replication type transposon, although Tn5 could be both a rolling replication and a cut and insert type transposon.

The present vectors may further comprise an insulator element located between the transposon insertion sequences and the multicloning site on the vector. In one embodiment, the insulator element is selected from the group consisting of an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, and a matrix attachment region element.

4. *Other Promoters and Enhancers*

The first promoter operably-linked to the transposase gene and the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter. Constitutive promoters include, but are not limited to, immediate early cytomegalovirus (CMV) promoter, herpes simplex virus 1 (HSV1) immediate early promoter, SV40 promoter, lysozyme promoter, early and late CMV promoters, early and late HSV promoters, β-actin promoter, tubulin promoter, Rous-Sarcoma virus (RSV) promoter, and heat-shock protein (HSP) promoter. Inducible promoters include tissue-specific promoters, developmentally-regulated promoters and chemically inducible promoters. Examples of tissue-specific promoters include the glucose-6-phosphatase (G6P) promoter, vitellogenin promoter, ovalbumin promoter, ovomucoid promoter, conalbumin promoter, ovotransferrin promoter, prolactin promoter, kidney uromodulin promoter, and placental lactogen promoter. The G6P promoter sequence may be deduced from a rat G6P gene untranslated upstream region provided in GenBank accession number U57552.1. Examples of developmentally-regulated promoters include the homeobox promoters and several hormone induced promoters. Examples of chemically inducible promoters include reproductive hormone

induced promoters and antibiotic inducible promoters such as the tetracycline inducible promoter and the zinc-inducible metallothionine promoter.

Other inducible promoter systems include the Lac operator repressor system inducible by IPTG (isopropyl beta-D-thiogalactoside) (Cronin, A. et al. 2001. Genes and Development, v. 15), ecdysone-based inducible systems (Hoppe, U. C. et al. 2000. Mol. Ther. 1:159-164); estrogen-based inducible systems (Braselmann, S. et al. 1993. Proc. Natl. Acad. Sci. 90:1657-1661); progesterone-based inducible systems using a chimeric regulator, GLVP, which is a hybrid protein consisting of the GAL4 binding domain and the herpes simplex virus transcriptional activation domain, VP16, and a truncated form of the human progesterone receptor that retains the ability to bind ligand and can be turned on by RU486 (Wang, et al. 1994. Proc. Natl. Acad. Sci. 91:8180-8184); CID-based inducible systems using chemical inducers of dimerization (CIDs) to regulate gene expression, such as a system wherein rapamycin induces dimerization of the cellular proteins FKBP12 and FRAP (Belshaw, P. J. et al. 1996. J. Chem. Biol. 3:731-738; Fan, L. et al. 1999. Hum. Gene Ther. 10:2273-2285; Shariat, S.F. et al. 2001. Cancer Res. 61:2562-2571; Spencer, D.M. 1996. Curr. Biol. 6:839-847). Chemical substances that activate the chemically inducible promoters can be administered to the animal containing the transgene of interest via any method known to those of skill in the art.

Other examples of cell-specific and constitutive promoters include but are not limited to smooth-muscle SM22 promoter, including chimeric SM22alpha/telokin promoters (Hoggatt A.M. et al., 2002. Circ Res. 91(12):1151-9); ubiquitin C promoter (Biochim Biophys Acta, 2003. Jan. 3;1625(1):52-63); Hsf2 promoter; murine COMP (cartilage oligomeric matrix protein) promoter; early B cell-specific mb-1 promoter (Sigvardsson M., et al., 2002. Mol. Cell Biol. 22(24):8539-51); prostate specific antigen (PSA) promoter (Yoshimura I. et al., 2002, J. Urol. 168(6):2659-64); exorh promoter and pineal expression-promoting element (Asaoka Y., et al., 2002. Proc. Natl. Acad. Sci. 99(24):15456-61); neural and liver ceramidase gene promoters (Okino N. et al., 2002. Biochem. Biophys. Res. Commun. 299(1):160-6); PSP94 gene promoter/enhancer (Gabril M.Y. et al., 2002. Gene Ther. 9(23):1589-99); promoter of the human FAT/CD36 gene (Kuriki C., et al., 2002. Biol. Pharm. Bull. 25(11):1476-8); VL30 promoter (Staplin W.R. et al., 2002. Blood October 24, 2002); and, IL-10 promoter (Brenner S., et al., 2002. J. Biol. Chem. December 18, 2002). Additional promoters are shown in Table 1.

Examples of avian promoters include, but are not limited to, promoters controlling expression of egg white proteins, such as ovalbumin, ovotransferrin (conalbumin), ovomucoid, lysozyme, ovomucin, g2 ovoglobulin, g3 ovoglobulin, ovoflavoprotein, ovostatin (ovomacroglobin), cystatin, avidin, thiamine-binding protein, glutamyl aminopeptidase minor

glycoprotein 1, minor glycoprotein 2; and promoters controlling expression of egg-yolk proteins, such as vitellogenin, very low-density lipoproteins, low density lipoprotein, cobalamin-binding protein, riboflavin-binding protein, biotin-binding protein (Awade, 1996. Z. Lebensm. Unters. Forsch. 202:1-14). An advantage of using the vitellogenin promoter is that it is active during the egg-laying stage of an animal's life-cycle, which allows for the production of the protein of interest to be temporally connected to the import of the protein of interest into the egg yolk when the protein of interest is equipped with an appropriate targeting sequence. In some embodiments, the avian promoter is an oviduct-specific promoter. As used herein, the term "oviduct-specific promoter" includes, but is not limited to, ovalbumin; ovotransferrin (conalbumin); ovomucoid; 01, 02, 03, 04 or 05 avidin; ovomucin; g2 ovoglobulin; g3 ovoglobulin; ovoflavoprotein; and ovostatin (ovomacroglobin) promoters.

When germline transformation occurs via cardiovascular, intraovarian or intratesticular administration, or when hepatocytes are targeted for incorporation of components of a vector through non-germ line administration, liver-specific promoters may be operably-linked to the gene of interest to achieve liver-specific expression of the transgene. Liver-specific promoters of the present invention include, but are not limited to, the following promoters, vitellogenin promoter, G6P promoter, cholesterol-7-alpha-hydroxylase (CYP7A) promoter, phenylalanine hydroxylase (PAH) promoter, protein C gene promoter, insulin-like growth factor I (IGF-I) promoter, bilirubin UDP-glucuronosyltransferase promoter, aldolase B promoter, furin promoter, metallothionine promoter, albumin promoter, and insulin promoter.

Also included in this invention are modified promoters/enhancers wherein elements of a single promoter are duplicated, modified, or otherwise changed. In one embodiment, steroid hormone-binding domains of the ovalbumin promoter are moved from about -3.5 kb to within approximately the first 1000 base pairs of the gene of interest. Modifying an existing promoter with promoter/enhancer elements not found naturally in the promoter, as well as building an entirely synthetic promoter, or drawing promoter/enhancer elements from various genes together on a non-natural backbone, are all encompassed by the current invention.

Accordingly, it is to be understood that the promoters contained within the transposon-based vectors of the present invention may be entire promoter sequences or fragments of promoter sequences. The constitutive and inducible promoters contained within the transposon-based vectors may also be modified by the addition of one or more modified Kozak sequences comprising any one of SEQ ID NOs:31 to 40.

As indicated above, the present invention includes transposon-based vectors containing one or more enhancers. These enhancers may or may not be operably-linked to their native

promoter and may be located at any distance from their operably-linked promoter. A promoter operably-linked to an enhancer and a promoter modified to eliminate repressive regulatory effects are referred to herein as an "enhanced promoter." The enhancers contained within the transposon-based vectors may be enhancers found in birds, such as an ovalbumin enhancer, but are not limited to these types of enhancers. In one embodiment, an approximately 675 base pair enhancer element of an ovalbumin promoter is cloned upstream of an ovalbumin promoter with 300 base pairs of spacer DNA separating the enhancer and promoter. In one embodiment, the enhancer used as a part of the present invention comprises base pairs 1-675 of a chicken ovalbumin enhancer from GenBank accession #S82527.1. The polynucleotide sequence of this enhancer is provided in SEQ ID NO:41.

Also included in some of the transposon-based vectors of the present invention are cap sites and fragments of cap sites. In one embodiment, approximately 50 base pairs of a 5' untranslated region wherein the capsite resides are added on the 3' end of an enhanced promoter or promoter. An exemplary 5' untranslated region is provided in SEQ ID NO:42. A putative cap-site residing in this 5' untranslated region preferably comprises the polynucleotide sequence provided in SEQ ID NO:43.

In one embodiment of the present invention, the first promoter operably-linked to the transposase gene is a constitutive promoter and the second promoter operably-linked to the gene of interest is a cell specific promoter. In the second embodiment, use of the first constitutive promoter allows for constitutive activation of the transposase gene and incorporation of the gene of interest into virtually all cell types, including the germline of the recipient animal. Although the gene of interest is incorporated into the germline generally, the gene of interest may only be expressed in a tissue-specific manner to achieve gene therapy. A transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered by any route, and in several embodiments, the vector is administered to the cardiovascular system, directly to an ovary, to an artery leading to the ovary or to a lymphatic system or fluid proximal to the ovary. In another embodiment, the transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered to vessels supplying the liver, muscle, brain, lung, kidney, heart or any other desired organ, tissue or cellular target. In another embodiment, the transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered to cells for culture *in vitro*.

It should be noted that cell- or tissue-specific expression as described herein does not require a complete absence of expression in cells or tissues other than the preferred cell or tissue.

Instead, "cell-specific" or "tissue-specific" expression refers to a majority of the expression of a particular gene of interest in the preferred cell or tissue, respectively.

When incorporation of the gene of interest into the germline is not preferred, the first promoter operably-linked to the transposase gene can be a tissue-specific or cell-specific promoter. For example, transfection of a transposon-based vector containing a transposase gene operably-linked to a liver specific promoter such as the G6P promoter or vitellogenin promoter provides for activation of the transposase gene and incorporation of the gene of interest in the cells of the liver *in vivo*, or *in vitro*, but not into the germline and other cells generally. In another example, transfection of a transposon-based vector containing a transposase gene operably-linked to an oviduct specific promoter such as the ovalbumin promoter provides for activation of the transposase gene and incorporation of the gene of interest in the cells of the oviduct *in vivo* or into oviduct cells *in vitro*, but not into the germline and other cells generally. In this embodiment, the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter. In one embodiment, both the first promoter and the second promoter are an ovalbumin promoter. In embodiments wherein tissue-specific expression or incorporation is desired, it is preferred that the transposon-based vector is administered directly to the tissue of interest, to the cardiovascular system which provides blood supply to the tissue of interest, to an artery leading to the organ or tissue of interest or to fluids surrounding the organ or tissue of interest. In one embodiment, the tissue of interest is the oviduct and administration is achieved by direct injection into the oviduct, into the cardiovascular system, or an artery leading to the oviduct. In another embodiment, the tissue of interest is the liver and administration is achieved by direct injection into the cardiovascular system, the portal vein or hepatic artery. In another embodiment, the tissue of interest is cardiac muscle tissue in the heart and administration is achieved by direct injection into the coronary arteries or left cardiac ventricle. In another embodiment, the tissue of interest is neural tissue and administration is achieved by direct injection into the cardiovascular system, the left cardiac ventricle, a cerebrovascular or spinovascular artery. In yet another embodiment, the target is a solid tumor and the administration is achieved by injection into a vessel supplying the tumor or by injection into the tumor.

Accordingly, cell specific promoters may be used to enhance transcription in selected tissues. In birds, for example, promoters that are found in cells of the fallopian tube, such as ovalbumin, conalbumin, ovomucoid and/or lysozyme, are used in the vectors to ensure transcription of the gene of interest in the epithelial cells and tubular gland cells of the fallopian tube, leading to synthesis of the desired protein encoded by the gene and deposition into the egg

white. In liver cells, the G6P promoter may be employed to drive transcription of the gene of interest for protein production. Proteins made in the liver of birds may be delivered to the egg yolk. Proteins made in transfected cells in vitro may be released into cell culture medium.

In order to achieve higher or more efficient expression of the transposase gene, the promoter and other regulatory sequences operably-linked to the transposase gene may be those derived from the host. These host specific regulatory sequences can be tissue specific as described above or can be of a constitutive nature.

Table 1

Reproductive tissue	Promoter	Ref.	Function/comments
testes, spermatogenesis	SPATA4	1	constitutive 30 d after birth in rat URE, Upstream Regulatory Element
placenta, glycoprotein	ERVWE1	2	is tissue spec. enhancer
breast epithelium and breast cancer	mammaglobin	6	specific to breast epithelium and cancer
prostate	EPSA	17	enhanced prostate-specific antigen promoter
testes	ATC	25	AlphaT-catenin specific for testes, skeletal, brain cardiomyocytes
prostate	PB	67	probasin promoter
Vision			
rod/cone	mCAR	3	cone photoreceptors and pinealocytes
retina	ATH5	15	functions in retinal ganglia and precursors
eye, brain	rhodopsin	27	
kertocytes	keratocan	42	specific to the corneal stroma
retina	RPE65	59	
Muscle			
vascular smooth muscle	TFPI	13	Tissue Factor Pathway Inhibitor – low level expression in endothelial and smooth muscle cells of vascular system
cardiac specific	MLC2v	14, 26	ventricular myosin light chain
cardiac	CAR3	18	BMP response element that directs cardiac specific expression

Reproductive tissue	Promoter	Ref.	Function/comments
skeletal	C5-12	22	high level, muscle spec expression to drive target gene
skeletal	AdmDys, AdmCTLA4Ig	32	muscle creatine kinase promoter
smooth muscle	PDE5A	41	chromosome 4q26, phosphodiesterase
smooth muscle	AlphaTM	45	use intronic splicing elements to restrict expression to smooth muscle vs skeletal
skeletal	myostatin	48	fiber type-specific expression of myostatin
Endocrine/nervous			
glucocorticoid	GR 1B-1E	4, 12	glucocorticoid receptor promoter/ all cells
neuroblastoma	M2-2	8, 36	M2 muscarinic receptor
brain	Abeta	16	amyloid beta-protein; 30 bp fragment needed for PC12 and glial cell expression
brain	enolase	21	neuron-specific; high in hippocampus, intermediate in cortex, low in cerebellum
synapses	rapsyn	29	clusters acetylcholine receptors at neuromuscular junction
neuropeptide precursor	VGF	39	express limited to neurons in central and peripheral nervous system and specific endocrine cells in adenohypophysis, adrenal medulla, GI tract and pancreas
mammalian nervous system	BMP/RA	46	use of methylation to control tissue specificity in neural cells.
central and peripheral noradrenergic neurons	Phox2a/Phox2b	47	regulation of neuron differentiation
brain	BAI1-AP4	55	spec to cerebral cortex and hippocampus
Gastrointestinal			
UDP glucoronsyltransferase	UGT1A7	11	gastric mucosa

Reproductive tissue	Promoter	Ref.	Function/comments
	UGT1A8	11	small intestine and colon
	UGT1A10	11	small intestine and colon
colon cancer	PKCbetaII	20	Protein kinase C betaII (PKCbetaII); express in colon cancer to selectively kill it.
Cancer			
tumor suppressor 4.1B	4.1B	5	2 isoforms, 1 spec to brain, 1 in kidney
nestin	nestin	63	second intron regulates tissue specificity
cancer spec promoter	hTRT/hSPA1	68	dual promoter system for cancer specificity
Blood/lymph system			
Thyroid	thyroglobulin	10	Thyroid spec. -- express to kill thyroid tumors
Thyroid	calcitonin	10	medullary thyroid tumors
Thyroid	GR 1A	12	regulation controlled by DREAM
thyroid	thyroglobulin	50	transcriptional repressor
arterial endothelial cells	ALK1	60	activin receptor-like kinase
Nonspecific			
RNA polymerase II		7	
gene silencing	Gnasx1, Nespas	31	
beta-globin	beta globin	53	
Cardiac	M2-1	8	M2 muscarinic receptor
Lung	hBD-2	19	IL-17 induced transcription in airway epithelium
pulmonary surfactant protein	SP-C	62	Alveolar type II cells
ciliated cell-specific prom	FOZJ1	70	use in ciliated epithelial cells for CF treatment
surfactant protein expression	SPA-D	73	Possible treatment in premature babies

Reproductive tissue	Promoter	Ref.	Function/comments
Clara cell secretory protein	CCSP	75	
Dental			
teeth/bone	DSPP	28	extracellular matrix protein dentin sialophosphoprotein
Adipose			
adipogenesis	EPAS1	33	endothelial PAS domain -- role in adipocyte differentiation
Epidermal			
differentiated epidermis	involucrin	38	
desmosomal protein	CDSN	58	stratum granulosum and stratum corneum of epidermis
Liver			
liver spec albumin	Albumin	49	
serum alpha-fetoprotein	AFP	56	liver spec regulation

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B. Methods of Transfecting Cells

1. *Transfection of LMH or LMH2A Cells in vitro*

DNA

IFN expression vector DNA (*e.g.*, any one of SEQ ID NOs:17-28) was prepared in either methylating or non-methylating bacteria, and was endotoxin-free. Agarose gels showed a single plasmid of the appropriate size. DNA was resuspended in molecular biology grade, sterile water at a concentration of at least 0.5 μg/μl. The concentration was verified by spectrophotometry, and the 260/280 ratio was 1.8 or greater. A stock of each DNA sample, diluted to 0.5 μg/μl in sterile, molecular biology grade water, was prepared in the cell culture lab, and this stock used for all transfections. When not in use, the DNA stocks were kept frozen at -30°C in small aliquots to avoid repeated freezing and thawing.

Transfection

The transfection reagent used for LMH or LMH2A cells was FuGENE 6 (Roche Applied Science). This reagent was used at a 1:6 ratio (μg of DNA: μl of transfection reagent) for all transfections in LMH or LMH2A cells. The chart below shows the amount of DNA and FuGENE 6 used for typical cell culture formats (T25 and T75 tissue culture flasks). If it is necessary to perform transfections in other formats, the amounts of serum free medium (SFM), FuGENE 6 and DNA are scaled appropriately based on the surface area of the flask or well used. The diluent (SFM) is any serum-free cell culture media appropriate for the cells, and it does not contain any antibiotics or fungicides.

Table 2

DNA:FuGENE = 1:6 [DNA]=0.5μg/μl

	T25	T75
SFM	250 μl	800 μl
FuGENE 6	12 μl	48 μl
DNA	4 μl	16 μl

Protocol

1. Cells used for transfection were split 24-48 hours prior to the experiment, so that they were actively growing and 50-80% confluent at the time of transfection.

2. FuGENE was warmed to room temperature before use. Because FuGENE is sensitive to prolonged exposure to air, the vial was kept tightly closed when not in use. The vial of FuGENE was returned to the refrigerator as soon as possible.

3. The required amount of FuGENE was pipetted into the SFM in a sterile microcentrifuge tube. The fluid was mixed gently but thoroughly, by tapping or flicking the tube, and incubated for 5 minutes at room temperature.

4. The required amount of DNA was added to the diluted FuGENE and mixed by vortexing for one second.

5. The mixture was incubated at room temperature for 1 hour.

6. During the incubation period, media on cells was replaced with fresh growth media. This media optionally contained serum, if needed, but did not contain antibiotics or fungicides unless absolutely required, as this can reduce the transfection efficiency.

7. The entire volume of the transfection complex was added to the cells. The flask was rocked to mix thoroughly.

8. The flasks were incubated at 37°C and 5% CO_2.

9. Cells were fed and samples obtained as required. After the first 24 hours, cells were optionally fed with media containing antibiotics and/or fungicides, if desired.

2. *Transfection of Other Cells*

The same methods described above for LMH and LMH2A cells are used for transfection of chicken tubular gland cells or other cell types such as Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, and human ARPT-19 cells.

C. Purification of Interferon Alpha 2b

The purification methods are described here with respect to IFN-α 2b, but the methods are similarly applicable to other interferons (*e.g.*, IFN-α 2a, IFN-β).

1. Media preparation

Media containing recombinant 3xFlag-IFN-α 2b produced by transfected cells was harvested and immediately frozen. Later the medium was thawed, filtered through a 0.45 micron cellulose acetate bottle-top filter to ensure that all particulate was removed prior to being loaded on the column.

2. Affinity Purification

The medium containing recombinant 3xFlag-IFN-α 2b produced by transfected cells was subjected to affinity purification using an Anti-Flag M2 Affinity Gel (Sigma, product code A2220) loaded onto a Poly-Prep Chromatography Column (BioRad, catalog 731-1550). A slurry of anti-Flag M2 gel was applied to Poly-Prep Chromatography Column, and the column was equilibrated at 1 ml/min with wash buffer (Tris Buffered Saline: 150 mM NaCl, 100 mM Tris, pH 7.5 (TBS)) for 30 column volumes. After equilibration was complete, the prepared medium containing 3xFlag-IFN from cultured and transfected cells was applied to the column.

The media sample passed through the column, and the column was washed for 10 column volumes with TBS. Next, 8 column volumes elution buffer (100 mM Tris, 0.5 M NaCl, pH 2.85) were run through the column, followed by 4 column volumes of TBS, and the eluent was collected. The eluent was immediately adjusted to a final pH of 8.0 with the addition of 1 M Tris, pH 8.0.

The eluent was transferred to an Amicon Ultra-15 (that was pre-washed with TBS) and centrifuged at 3,500 x g until the sample was concentrated to the desired volume.

3. Size exclusion chromatography

The concentrated eluent from the affinity purification procedure was then subjected to size exclusion chromatography as a final polishing step in the purification procedure. First, a superdex 75 10/300 GL column (GE Healthcare) was equilibrated with TBS. Multiple size exclusion runs were done in which a sample volume of 400 µl for each run was passed over the column. Fractions containing 3xFlag-IFN from each run were then pooled, transferred to an Amicon Ultra-15, and concentrated to the desired final volume.

The purification procedure was evaluated at various stages using a sandwich ELISA assay (See section D.1. below). SDS-PAGE analysis with subsequent Coomassie blue staining was done to indicate both molecular weight and purity of the purified 3xFlag-IFN (See section D.2. below).

D. Interferon Alpha 2b Detection

1. *Interferon Alpha 2b (IFN-α 2b) Measurement with ELISA*

IFN-α 2b was measured using the following sandwich ELISA protocol:

1. Diluted monoclonal anti-IFN-α 2b (Abcam, Cat. #ab9388) 1:1000 in 2x-carbonate, pH 9.6 such that the final working dilution concentration is 2 μg/mL. This same antibody also recognizes IFN-α 2a.

2. Added 100 μL of the diluted antibody into to the appropriate wells of the ELISA plate.

3. Allowed 96-well plate to coat overnight at 4°C or for 1 hour at 37°C.

4. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).

5. Transferred 200 μL of blocking buffer (1.5% bovine serum albumen (BSA)/1x TBS/0.05% TWEEN) to the appropriate wells of the ELISA plate and allowed 96-well plate to block overnight @ 4°C or for 45 minutes at room temperature.

6. Diluted the purified fusion 3xFlag-IFN-α 2b standard (clone #206) in negative control media (5% FCS/Waymouth, Gibco) such that the final working dilution concentration is 16 ng/mL.

7. Diluted test samples in negative control media (5% FCS/Waymouth , Gibco).

8. Removed the blocking buffer by manually "flicking" the ELISA plate into the sink.

9. Added the diluted samples and fusion protein standards into 96-well plate and incubate the ELISA plate at room temperature for 1 hour.

10. Diluted fresh Anti FLAG M2 Alkaline Phosphatase Antibody 1:8,000 (Sigma, Cat. # A9469) such that the final working dilution concentration is 125 ng/mL.

11. Added 100 μL of the diluted antibody into to the appropriate wells of the ELISA plate.

12. Incubated the ELISA plate at room temperature for 1 hour.

13. Diluted the p-nitrophenyl phosphate substrate solution in 1X diethanolamine (DEA) substrate buffer, pH 9.8 (KPL, Cat.# 50-80-02) such that the final working dilution concentration is 1 mg/mL.

14. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).

15. Added 100 μL of the diluted p-nitrophenyl phosphate substrate solution to the appropriate wells of the ELISA plate

16. Using plate reader, took the absorbance readings at 405 nm of the ELISA plate at 30, 60, 90, and 120 minute intervals.

Culture medium was applied to the ELISA either in an undiluted or slightly diluted manner. 3xFlag-IFN-α 2b was detected in this assay. The 3xFlag-IFN-α 2b levels were

determined by reference to the 3xFlag-IFN-α 2b standard curve and are presented in various figures throughout this application.

The purification procedure was evaluated at various stages using a sandwich ELISA assay (See section D.1. above). SDS-PAGE analysis with subsequent Coomassie blue staining or Western blotting was done to indicate both molecular weight and purity of the purified 3xFlag-IFN (See section D.2. below).

2. *Detection of Interferon Alpha 2b Expression with Immunoblotting*

SDS-PAGE:

Sample mixtures, including negative control media, were heated for 8 minutes at 100°C and loaded onto a 10-20% Tris-HCl gel. The samples were run at 200 V for 1 hour 10 minutes in Tris-Glycine-SDS buffer.

3x-Flag detection:

1. The finished gel was placed into the Western blot transfer buffer for 2 minutes. This equilibrated the gel in the buffer used for the transfer.
2. The gel was rehydrated for 1 minute in Western blot transfer buffer. A sheet of nitrocellulose paper was cut to the exact size of the gel to be transferred.
3. The electrophoretic transfer was occurred for 50 minutes at 100 V.
4. The blot was removed from the transfer apparatus and blocked with 5.0% milk in TBS/TWEEN 20. Blocking was allowed for 1 hour at 37°C.
5. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.
6. The blot was incubated in Anti-FLAG M2 (Sigma, Cat. # A9469) conjugated with alkaline phosphatase diluted appropriately 1:5,000 with 1% gelatin in TBS/TWEEN 20 for 1 hour at room temperature.
7. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.
8. Antibody bound to antigen was detected by using the BCIP/NBT Liquid Substrate System (KPL). The substrate solution was applied until color was detected (5-10 minutes).
9. Color formation (enzyme reaction) was stopped by rinsing blots with distilled H_2O.
10. The blot was air-dried on paper towel.

Interferon detection:

1. The interferon also could be detected directly with an anti-interferon antibody as follows. The finished gel was placed into the Western blot transfer buffer for 2 minutes. This equilibrated the gel in the buffer used for the transfer.

2. The gel was rehydrated for 1 minute in Western blot transfer buffer. A sheet of nitrocellulose paper was cut to the exact size of the gel to be transferred.

3. The electrophoretic transfer was occurred for 50 minutes at 100 V.

4. The blot was removed from the transfer apparatus and was blocked with 5.0% MILK in TBS/TWEEN 20. Blocking was allowed for 1 hour at 37 °C.

5. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.

6. The blot was incubated in monoclonal anti-IFN-α 2b (abcam, Cat # ab9388) diluted appropriately 1:2,000 with 1% gelatin in TBS/TWEEN 20 for 1 hour at room temperature.

7. The blot was washed three times for 5 minutes per wash in TBS/TWEEN 20.

8. The blot was incubated in anti-mouse IgG (abcam, Cat # ab6729) conjugated with alkaline phosphatase diluted appropriately 1:10,000 with 1% gelatin in TBS/TWEEN 20 for 1 hour at room temperature.

9. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.

10. Antibody bound to antigen was detected by using the 5-bromo,4-chloro,3-indolylphosphate (BCIP)/ nitrobluetetrazolium (NBT) Liquid Substrate System (KPL). The substrate solution was applied until color was detected (5-10 minutes).

11. Color formation (enzyme reaction) was stopped by rinsing blots with dH_2O.

12. The blot was air-dried on a paper towel.

3. *Vectors for Interferon Alpha 2b Production*

The vectors of the present invention employ some of the vector components (backbone vectors and promoters) described in the previous sections and also include the multiple cloning site (MCS) comprising the gene of interest. In one embodiment, the gene of interest encodes for a human interferon. In certain embodiments, the gene of interest encodes a human IFN-α 2a, IFN-α 2b, or IFN-β1a protein. The following vectors, SEQ ID NOs:17 through 28, all contain a gene of interest encoding a human interferon protein:

(SEQ ID NO:17): #188 HS4 Flanked Backbone Vector (CMVep-Intron A + hIFN-α 2b)

(SEQ ID NO:18): #206 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + 3xFlag-hIFN-α 2b)

(SEQ ID NO:19): #207 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 2 (SEQ ID NO:15) + hIFN-α 2b)

(SEQ ID NO:20) #261 pTn10-Gen/Mar BV Vector (#5022) (CMV.Ovalp vs. 1 (SEQ ID NO:14)/mature hIFN-α 2b/OvpolyA)

(SEQ ID NO:21) #262 pTn10-Gen/Mar BV Vector (#5022) (CMV.Ovalp vs. 1 (SEQ ID NO:14)/ 3xFlag/hIFN-α 2b/OvpolyA)

(SEQ ID NO:22) #248 TnPuroMAR Flanked Backbone Vector (#5021) (Hybrid promoter vs 1 (SEQ ID NO:14)/hIFN-α 2b/Syn PolyA)

(SEQ ID NO:23) #309 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + IFN-α 2b with native signal sequence)

(SEQ ID NO:24) #310 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + 3xFlag IFN-α 2b with encoded N-linked glycosylation site)

(SEQ ID NO:25) #311 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + mature IFN-α 2b with encoded N-linked glycosylation site)

(SEQ ID NO:26) #313 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + mature IFN-α 2a)

(SEQ ID NO:27) #286 Codon optimized IFN-α 2a TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + 3xFlag IFN-α 2a)

(SEQ ID NO:28) #295 TnPuroMAR Flanked Backbone Vector (#5021) (hybrid promoter version 1 (SEQ ID NO:14) + mature IFN-α 2a)

E. Methods of *In Vivo* Administration

The polynucleotide cassettes may be delivered through the vascular system to be distributed to the cells supplied by that vessel. For example, the compositions may be administered through the cardiovascular system to reach target tissues and cells receiving blood supply. In one embodiment, the compositions may be administered through any chamber of the heart, including the right ventricle, the left ventricle, the right atrium or the left atrium. Administration into the right side of the heart may target the pulmonary circulation and tissues supplied by the pulmonary artery. Administration into the left side of the heart may target the systemic circulation through the aorta and any of its branches, including but not limited to the coronary vessels, the ovarian or testicular arteries, the renal arteries, the arteries supplying the gastrointestinal and pelvic tissues, including the celiac, cranial mesenteric and caudal mesenteric vessels and their branches, the common iliac arteries and their branches to the pelvic organs, the gastrointestinal system and the lower extremity, the carotid, brachiocephalic and subclavian arteries. It is to be understood that the specific names of blood vessels change with the species under consideration and are known to one of ordinary skill in the art. Administration into the left ventricle or ascending or descending aorta supplies any of the tissues receiving blood supply from the aorta and its branches, including but not limited to the testes, ovary, oviduct, and liver.

Germline cells and other cells may be transfected in this manner. For example, the compositions may be placed in the left ventricle, the aorta or directly into an artery supplying the ovary or supplying the fallopian tube to transfect cells in those tissues. In this manner, follicles could be transfected to create a germline transgenic animal. Alternatively, supplying the compositions through the artery leading to the oviduct would preferably transfect the tubular gland and epithelial cells. Such transfected cells could manufacture a desired protein or peptide for deposition in the egg white. Administration of the compositions through the left cardiac ventricle, the portal vein or hepatic artery would target uptake and transformation of hepatic cells. Administration may occur through any means, for example by injection into the left ventricle, or by administration through a cannula or needle introduced into the left atrium, left ventricle, aorta or a branch thereof.

Intravascular administration further includes administration in to any vein, including but not limited to veins in the systemic circulation and veins in the hepatic portal circulation. Intravascular administration further includes administration into the cerebrovascular system, including the carotid arteries, the vertebral arteries and branches thereof.

Intravascular administration may be coupled with methods known to influence the permeability of vascular barriers such as the blood brain barrier and the blood testes barrier, in order to enhance transfection of cells that are difficult to affect through vascular administration. Such methods are known to one of ordinary skill in the art and include use of hyperosmotic agents, mannitol, hypothermia, nitric oxide, alkylglycerols, lipopolysaccharides (Haluska et al., Clin. J. Oncol. Nursing 8(3): 263-267, 2004; Brown et al., Brain Res., 1014: 221-227, 2004; Ikeda et al., Acta Neurochir. Suppl. 86:559-563, 2004; Weyerbrock et al., J. Neurosurg. 99(4):728-737, 2003; Erdlenbruch et al., Br. J. Pharmacol. 139(4):685-694, 2003; Gaillard et al., Microvasc. Res. 65(1):24-31, 2003; Lee et al., Biol. Reprod. 70(2):267-276, 2004)).

Intravascular administration may also be coupled with methods known to influence vascular diameter, such as use of beta blockers, nitric oxide generators, prostaglandins and other reagents that increase vascular diameter and blood flow.

Administration through the urethra and into the bladder would target the transitional epithelium of the bladder. Administration through the vagina and cervix would target the lining of the uterus and the epithelial cells of the fallopian tube.

The polynucleotide cassettes may be administered in a single administration, multiple administrations, continuously, or intermittently. The polynucleotide cassettes may be administered by injection, via a catheter, an osmotic mini-pump or any other method. In some

embodiments, a polynucleotide cassette is administered to an animal in multiple administrations, each administration containing the polynucleotide cassette and a different transfecting reagent.

In a preferred embodiment, the animal is an egg-laying animal, and more preferably, an avian, and the transposon-based vectors comprising the polynucleotide cassettes are administered into the vascular system, preferably into the heart. The vector may be injected into the venous system in locations such as the jugular vein and the metatarsal vein. In one embodiment, between approximately 1 and 1000 μg, 1 and 200 μg, 5 and 200 μg, or 5 and 150 μg of a transposon-based vector containing the polynucleotide cassette is administered to the vascular system, preferably into the heart. In a chicken, it is preferred that between approximately 1 and 300 μg, or 5 and 200 μg are administered to the vascular system, preferably into the heart, more preferably into the left ventricle. The total injection volume for administration into the left ventricle of a chicken may range from about 10 μl to about 5.0 ml, or from about 100 μl to about 1.5 ml, or from about 200 μl to about 1.0 ml, or from about 200 μl to about 800 μl. It is to be understood that the total injection volume may vary depending on the duration of the injection. Longer injection durations may accommodate higher total volumes. In a quail, it is preferred that between approximately 1 and 200 μg, or between approximately 5 and 200 μg are administered to the vascular system, preferably into the heart, more preferably into the left ventricle. The total injection volume for administration into the left ventricle of a quail may range from about 10 μl to about 1.0 ml, or from about 100 μl to about 800 μl, or from about 200 μl to about 600 μl. It is to be understood that the total injection volume may vary depending on the duration of the injection. Longer injection durations may accommodate higher total volumes. The microgram quantities represent the total amount of the vector with the transfection reagent.

In another embodiment, the animal is an egg-laying animal, and more preferably, an avian. In one embodiment, between approximately 1 and 150 μg, 1 and 100 μg, 1 and 50 μg, preferably between 1 and 20 μg, and more preferably between 5 and 10 μg of a transposon-based vector containing the polynucleotide cassette is administered to the oviduct of a bird. In a chicken, it is preferred that between approximately 1 and 100 μg, or 5 and 50 μg are administered. In a quail, it is preferred that between approximately 5 and 10 μg are administered. Optimal ranges depending upon the type of bird and the bird's stage of sexual maturity. Intraoviduct administration of the transposon-based vectors of the present invention result in a PCR positive signal in the oviduct tissue, whereas intravascular administration results in a PCR positive signal in the liver, ovary and other tissues. In other embodiments, the polynucleotide cassettes is administered to the cardiovascular system, for example the left cardiac ventricle, or directly into an artery that supplies the oviduct or the liver. These methods of administration may

also be combined with any methods for facilitating transfection, including without limitation, electroporation, gene guns, injection of naked DNA, and use of dimethyl sulfoxide (DMSO). U.S. Patent No. 7,527,966, U.S. Publication No. 2008-0235815, and PCT Publication No. WO 2005/062881 are hereby incorporated by reference in their entirety.

In specific embodiments, the disclosed backbone vectors are defined by the following annotations:

SEQ ID NO:1 (pTnMCS (base vector, without MCS extension) Vector #5001

Bp 1 – 130	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp1-130
Bp 133 – 1812	CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems) bp229-1873
Bp 1813 – 3018	Transposase, modified from Tn10 (GeneBank accession #J01829)Bp 108-1316
Bp 3019 – 3021	Engineered stop codon
Bp 3022 – 3374	Non-coding DNA from vector pNK2859
Bp 3375 – 3417	Lambda DNA from pNK2859
Bp 3418 – 3487	70bp of IS10 left from Tn10
Bp 3494 – 3700	Multiple cloning site from pBluescriptII sk(-), thru the XmaI site Bp 924-718
Bp 3701 – 3744	Multiple cloning site from pBluescriptII sk(-), from the XmaI site thru the XhoI site. These base pairs are usually lost when cloning into pTnMCS. Bp 717-673
Bp 3745 – 4184	Multiple cloning site from pBluescriptII sk(-), from the XhoI site bp 672-235
Bp 4190 – 4259	70 bp of IS10 from Tn10
Bp 4260 – 4301	Lambda DNA from pNK2859
Bp 4302 – 5167	Non-coding DNA from pNK2859
Bp 5168 – 7368	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:2 pTnX-MCS (Vector #5005) pTNMCS (base vector) with MCS extension

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) Bp 4-135
Bp 133 – 1785	CMV Promoter/Enhancer from vector pGWIZ (Gene Therapy Systems)
Bp 1786 – 3018	Transposase, modified from Tn10 (GeneBank accession #J01829) Bp 81-1313
Bp 3019 – 3021	Engineered stop codon

Bp 3022 – 3374	Non-coding DNA from vector pNK2859
Bp 3375 – 3416	Lambda DNA from pNK2859
Bp 3417 – 3486	70bp of IS10 left from Tn10 (GeneBank accession #J01829 Bp 1-70)
Bp 3487 – 3704	Multiple cloning site from pBluescriptII sk(-), thru XmaI
Bp 3705 – 3749	Multiple cloning site from pBluescriptII sk(-), from XmaI thru XhoI
Bp 3750 – 3845	Multiple cloning site extension from XhoI thru PspOMI
BP 3846 - 4275	Multiple cloning site from pBluescriptII sk(-), from PspOMI
Bp 4276 – 4345	70 bp of IS10 from Tn10 (GeneBank accession #J01829 Bp 70-1)
Bp 4346 – 4387	Lambda DNA from pNK2859
Bp 4388 – 5254	Non-coding DNA from pNK2859
Bp 5255 – 7455	pBluescriptII sk(-) base vector (Stratagene, INC) Bp 761-2961

SEQ ID NO:3 HS4 Flanked BV (Vector #5006)

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) Bp 4-135
Bp 133 – 1785	CMV Promoter/Enhancer from vector pGWIZ (Gene Therapy Systems) Bp 229-1873, including the combination of 2 NruI cut sites
Bp 1786 – 3018	Transposase, modified from Tn10 (GeneBank accession #J01829) Bp 81-1313
Bp 3019 – 3021	Engineered stop codon
Bp 3022 – 3374	Non-coding DNA from vector pNK2859
Bp 3375 – 3416	Lambda DNA from pNK2859
Bp 3417 – 3490	70bp of IS10 left from Tn10 (GeneBank accession #J01829 Bp 1-70)
Bp 3491 – 3680	Multiple cloning site from pBluescriptII sk(-), thru NotI Bp 926-737
Bp 3681 – 4922	HS4 – Beta-globin Insulator Element from Chicken gDNA
Bp 4923 – 5018	Multiple cloning site extension XhoI thru MluI
Bp 5019 – 6272	HS4 – Beta-globin Insulator Element from Chicken gDNA
Bp 6273 – 6342	70 bp of IS10 from Tn10 (GeneBank accession #J01829 Bp 70-1)
Bp 6343 – 6389	Lambda DNA from pNK2859
Bp 6390 – 8590	pBluescriptII sk(-) base vector (Stratagene, INC) Bp 761-2961

SEQ ID NO:4	pTn-10 HS4 Flanked Backbone (Vector #5012)
Bp 1-132	Remaining of F1 (-) Ori from pBluescript II sk(-)(Statagene Bp 4-135).

Bp 133-1806	CMV Promoter / Enhancer from vector pGWIZ (Gene Therapy Systems) Bp. 229-1873.
Bp 1807-3015	Tn-10 transposase, from pNK2859 (GeneBank accession #J01829 Bp. 81-1313).
Bp 3016-3367	Non-coding DNA, possible putative poly A, from vector pNK2859.
Bp 3368-3410	Lambda DNA from pNK2859.
Bp 3411-3480	70bp of IS 10 left from Tn10 (GeneBank accession #J01829 bp. 1-70
Bp 3481-3674	Multiple cloning site from pBluescript II sk(-), thru NotI Bp. 926-737.
Bp 3675-4916	Chicken Beta Globin HS4 Insulator Element (Genbank accession #NW_060254.0).
Bp 4917-5012	Multiple cloning site extension Xho I thru Mlu I.
Bp 5013-6266	Chicken Beta Globin HS4 Insulator Element (Genbank accession #NW_060254.0).
Bp 6267-6337	70bp of IS 10 left from Tn10 (GeneBank accession #J01829 bp. 1-70
Bp 6338-6382	Lambda DNA from pNK2859.
Bp 6383-8584	pBluescript II sk(-) Base Vector (Stratagene, Inc. Bp. 761-2961).

SEQ ID NO:5 pTN-10 MAR Flanked BV (Vector 5018)

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)

Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 5367	Lysozyme Matrix Attachment Region (MAR)
Bp 5368 – 5463	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI
Bp 5464 - 7168	Lysozyme Matrix Attachment Region (MAR)
Bp 7169 – 7238	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 7239 - 7281	Lambda DNA from pNK2859
Bp 7282 - 9486	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:6 (Vector 5020 pTN-10 PURO – LysRep2 Flanked BV)

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3484	Synthetic DNA added during construction
Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 4608	Lysozyme Rep2 from gDNA (corresponds to Genbank Accession #NW_060235)
Bp 4609 – 4686	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
Bp 4687 - 4999	HSV-TK polyA from pS65TC1 bp 3873-3561
Bp 5000 - 5028	Excess DNA from pMOD PURO (invivoGen)
BP 5029 - 5630	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116

Bp 5631 - 6016	SV40 promoter from pS65TC1, bp 2232-2617
Bp 6017 - 6022	MluI RE site
Bp 6023 - 6956	Lysozyme Rep2 from gDNA (corresponds to Genbank Accession #NW_060235)
Bp 6957 – 6968	Synthetic DNA added during construction including a PspOMI RE site
Bp 6969 – 7038	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 7039 - 7081	Lambda DNA from pNK2859
Bp 7082 - 7085	Synthetic DNA added during construction
Bp 7086 - 9286	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:7 (Vector #5019 pTN-10 PURO – HS4 Flanked BV)

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3484	Synthetic DNA added during construction
Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 4916	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
Bp 4917 – 4994	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
Bp 4995 - 5307	HSV-TK polyA from pS65TC1 bp 3873-3561

Bp 5308 - 5336	Excess DNA from pMOD PURO (invivoGen)
BP 5337 - 5938	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
Bp 5939 - 6324	SV40 promoter from pS65TC1, bp 2232-2617
Bp 6325 - 6330	MluI RE site
Bp 6331 - 7572	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
Bp 7573 – 7584	Synthetic DNA added during construction including a PspOMI RE site
Bp 7585 – 7654	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 7655 - 7697	Lambda DNA from pNK2859
Bp 7698 - 7701	Synthetic DNA added during construction
Bp 7702 - 9902	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:8 Vector #5021 pTN-10 PURO - MAR Flanked BV

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)
Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 5367	Lysozyme Matrix Attachment Region (MAR)
Bp 5368 – 5445	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
Bp 5446 - 5758	HSV-TK polyA from pS65TC1 bp 3873-3561

	BP 5759 - 6389	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 6390 - 6775	SV40 promoter from pS65TC1, bp 2232-2617
	Bp 6776 - 8486	Lysozyme Matrix Attachment Region (MAR)
	Bp 8487 – 8556	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
5	Bp 8557 - 8599	Lambda DNA from pNK2859
	Bp 8600 - 10804	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:9 (Vector #5022; pTN-10 Gen – MAR Flanked BV)

	Bp 1 – 5445	pTN-10 MAR Flanked BV, ID #5018
10	Bp 5446 - 5900	HSV-TK polyA from Taken from pIRES2-ZsGreen1, bp 4428-3974
	Bp 5901 - 6695	Kanamycin/Neomycin (G418) resistance gene, taken from pIRES2-ZsGreen1, Bp 3973-3179
	Bp 6696 - 7046	SV40 early promoter/enhancer taken from pIRES2-ZsGreen1,bp 3178-2828
15	Bp 7047 - 7219	Bacterial promoter for expression of KAN resistance gene, taken from pIRES2-ZsGreen1,bp 2827-2655
	Bp 7220 - 11248	pTN-10 MAR Flanked BV, bp 5458-9486

	SEQ ID NO:10	pTN-10 MAR Flanked BV Vector #5024
20	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 155 - 229	CMV promoter (from vector pGWIZ, Gene Therapy Systems bp 844-918
	Bp 230 - 350	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
25	Bp 351 - 1176	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1177 - 1184	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
	Bp 1185 – 1213	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1214 – 2422	Transposon, modified from Tn10 GenBank Accession #J01829 bp 108-1316
30		
	Bp 2423 – 2774	Putative PolyA from vector pNK2859
	Bp 2775 – 2817	Lambda DNA from pNK2859
	Bp 2818 – 2887	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)

Bp 2888 – 3058 pBluescriptII sk(-) base vector (Stratagene, INC) Bp 3059 - 3081 Multiple cloning site from pBluescriptII sk(-)thru NotI,

Bp 3082 - 4774 Chicken 5' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408

Bp 4775 – 4870 Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI

Bp 4871 - 6575 Chicken 3' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408

Bp 6576 – 6645 70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)

Bp 6646 - 6688 Lambda DNA from pNK2859

Bp 6689 - 8893 pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:11 Vector #5025 pTN-10 (-CMV Enh.)PURO - MAR Flanked BV

Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135

Bp 133 – 154 pGWIZ base vector (Gene Therapy Systems) bp 229-244

Bp 155 - 229 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)

Bp 230 - 350 CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)

Bp 351 - 1176 CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)

Bp 1177 - 1184 CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)

Bp 1185 – 1213 TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107

Bp 1214 – 2422 Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316

Bp 2423 – 2774 Putative PolyA from vector pNK2859

Bp 2775 – 2817 Lambda DNA from pNK2859

Bp 2818 – 2887 70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)

Bp 2888 – 3058 pBluescriptII sk(-) base vector (Stratagene, INC)

Bp 3059 - 3081 Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737

Bp 3082 - 4774 Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408

Bp 4775 – 4852 Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI

Bp 4853 - 5165 HSV-TK polyA from pS65TC1 bp 3873-3561

BP 5166 - 5796 Puromycin resistance gene from pMOD PURO (invivoGen)

Bp 5797 - 6182	SV40 promoter from pS65TC1, bp 2232-2617
Bp 6183 - 7893	Lysozyme Matrix Attachment Region (MAR)
Bp 7894 – 7963	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 7964 - 8010	Lambda DNA from pNK2859
Bp 8011 - 10211	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:12 Vector #5026 pTN-10 MAR Flanked BV #5026

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 155 - 540	SV40 promoter from pS65TC1 bp 2232-2617
Bp 541 - 661	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 662 - 1487	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1488 - 1495	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1496 – 1524	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1525 – 2733	Transposon, modified from Tn10 GenBank Accession #J01829 bp 108-1316
Bp 2734 – 3085	Putative PolyA from vector pNK2859
Bp 3086 – 3128	Lambda DNA from pNK2859
Bp 3129 – 3198	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3199 – 3369	pBluescriptII sk(-) base vector (Stratagene, INC)
Bp 3370 - 3392	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3393 - 5085	Chicken 5' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
Bp 5086 – 5181	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI
Bp 5182 - 6886	Chicken 3' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
Bp 6887 – 6956	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 6957 - 6999	Lambda DNA from pNK2859
Bp 7000 - 9204	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:13 pTN-10 SV 40 Pr.PURO - MAR Flanked BV Vector #5027

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene)bp 4-135
Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 155 - 540	SV40 Promoter from pS65TC1, Bp 2232-2617
Bp 541 - 661	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 662 - 1487	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1488 - 1495	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1496 – 1524	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1525 – 2733	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 2734 – 3085	Putative PolyA from vector pNK2859
Bp 3086 – 3128	Lambda DNA from pNK2859
Bp 3129 – 3198	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3199 – 3369	pBluescriptII sk(-) base vector (Stratagene, INC)
Bp 3370 - 3392	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3393 - 5085	Lysozyme Matrix Attachment Region (MAR) from chicken gDNA GenBank Accession #X98408.
Bp 5086 – 5163	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
Bp 5164 - 5476	HSV-TK polyA from pS65TC1 bp 3873-3561
BP 5477 - 6107	Puromycin resistance gene from pMOD PURO (invivoGen)
Bp 6108 - 6499	SV40 promoter from pS65TC1, bp 2232-2617
Bp 6500 - 8204	Lysozyme Matrix Attachment Region (MAR)
Bp 8205 – 8274	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 8275 - 8317	Lambda DNA from pNK2859
Bp 8318 - 10522	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

In specific embodiments, the disclosed hybrid promoters are defined by the following annotations:

SEQ ID NO:14 (CMV/Oval promoter Version 1 = ChOvp/CMVenh/CMVp)

Bp 1 - 840:	corresponds to bp 421-1260 from the chicken ovalbumin promoter, GenBank accession number

Bp 841- 1439: CMV Enhancer bp 245-843 taken from vector pGWhiz CMV promoter and enhancer bp 844-918 taken from vector pGWhiz (includes the CAAT box at 857-861 and the TATA box at 890-896).

Bp 1440 – 1514 CMV promoter

SEQ ID NO:15 (CMV/Oval promoter Version 2 = ChSDRE/CMVenh/ChNRE/CMVp)

Bp 1 - 180: Chicken steroid dependent response element from ovalbumin promoter

Bp 181 - 779: CMV Enhancer bp 245-843 taken from vector pGWhiz

Bp 780 - 1049: Chicken ovalbumin promoter negative response element

Bp 1050-1124: CMV promoter bp 844-918 taken from vector pGWhiz (includes the CAAT box at 857-861 and the TATA box at 890-896. Some references overlap the enhancer to different extents.)

In specific embodiments, the disclosed expression vectors are defined by the following annotations:

SEQ ID NO:17 Vector #188 Puro HFBV (CMVnpiA'/Conss/n3xf/hIFN-α2b/SynpyA)

Bp 1 - 4928 Puro HFBV (bp 1-4928)

Bp 4929 - 6572 CMVnpiA' (bp 245-1873 of gWIZ blank vector); includes CMV enhancer, promoter, Immediate-Early gene, EXON 1, CMV Intron A, CMV Immediate-Early gene, partial EXON 2

Bp 6573 - 6578 Synthetic DNA added during vector construction; Sal I cut site

Bp 6579 - 6641 Chicken Conalbumin Signal Sequence + Kozak sequence (6579-6585) (from GenBank Accession # X02009)

Bp 6642 - 6647 Synthetic DNA added during vector construction; BsrFI Cut site

Bp 6648 - 6698 3xFlag

Bp 6699 - 6713 Enterokinase Cleavage Site

Bp 6714 - 7211 Human Interferon alpha-α2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted

Bp 7212 - 7627 Synthetic polyA DNA; taken from gWIZ blank vector (bp 1921-2334)

Bp 7628 - 12631 Puro HFBV (bp 4929-9926)

SEQ ID NO:18 Vector #206

pTN-10 PURO MAR BV (CMV.Ovalp vs. 1/hIFNA/SynpyA)

Bp 1 - 5381 pTN-10 PURO MAR BV (bp 1-5381)

Bp 5382 - 6222	Chicken Ovalbumin Promoter (bp 1090-1929)
Bp 6223 - 6228	Synthetic DNA added during vector construction (EcoRI cut site used for ligation)
Bp 6229 - 6883	CMV enhancer/promoter (bp 245-899 of gWIZ blank vector)
Bp 6884 - 6905	XhoI site + bp 900-918 of CMVpromoter from gWIZ blank vector (from D.H. Clone 10; she used this site to add on the CMViA')
Bp 6906 - 7860	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate- early gene, partial Exon 2)
Bp 7861 - 7866	Synthetic DNA added during vector construction (SalI site used for ligation)
Bp 7867 - 7929	Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7873) (from GenBank Accession # X02009)
Bp 7930 - 7935	Synthetic DNA added during vector construction (BsrFI cut site used for ligation)
Bp 7936 - 7986	3x flag
Bp 7987 - 8001	Enterokinase Cleavage Site
Bp 8002 - 8499	Human Interferon alpha-2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted
Bp 8500 - 8505	Synthetic DNA added during vector construction (BamHI site used for ligation)
Bp 8506 - 8902	Synthetic polyA; taken from gWIZ blank vector (bp 1921- 2334)
Bp 8903 – 14322	pTN-10 PURO MAR BV (bp 5385-10804)

SEQ ID NO:19 Vector #207

pTN-10 PURO MAR BV (CMV.Ovalp vs. 2/Hifn-α 2b/SynpyA)

Bp 1 - 5381	pTN-10 PURO MAR BV (bp 1-5381)
Bp 5382 - 5567	Chicken SDRE (from ChOVep, bp 1100-1389) with EcroRI site at 3' end for ligations
Bp 5568 - 6172	CMVenhancer (from gWIZ blank vector, bp 245-843) with NgoMIV site at 3' end for ligations
Bp 6173 - 6448	Chicken NRE (from ChOvep, bp 1640-1909) with KpnI site at 3' end for ligations

Bp 6449 - 6526	CMVpromoter (from gWIZ blank vector, bp 844-915); has XhoI site (inserted "CTC" at bp 6505 to create XhoI site to ligate clone 10 to CMViA')
Bp 6527 - 7487	CMV Intron A' (CMV immediate early gene, exon 1; CMV Intron A; CMV immediate early gene, partial exon 2); from gWIZ blank vector bp 919-1873, with SalI site at 3' end for ligation
Bp 7488 - 7556	Chicken Conalbumin Signal Sequence + Kozak sequence (7488-7494) from GenBank Accession # X02009) with BsrFI site at 3' end for ligation
Bp 7557 - 7607	New 3x Flag
Bp 7608 - 7622	Enterokinase Cleavage Site
Bp 7623 - 8126	Human Interferon alpha-α 2b (IFN-α 2b) gene with BamHI site at 3' end for ligations; taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted
Bp 8127 - 8523	Synthetic polyA; taken from gWIZ blank vector (bp 1921-2334)
Bp 8524 – 13943	pTN-10 PURO MAR BV (bp 5385-10804)

SEQ ID NO:20 Vector 261 (pTn10-Gen/Mar BV (CMV.Ovalp vs. 1/mature hIFN-α 2b/OvpyA)

Bp 1 - 5381	pTn10-Gen/Mar BV (Bp 1-5381)
Bp 5382 - 6222	Chicken Ovalbumin Promoter (bp 1090-1929)
Bp 6223 6228	Synthetic DNA added during vector construction (EcoRI cut site used for ligation)
Bp 6229 - 6883	CMV enhancer/promoter (bp 245-899 of gWIZ blank vector)
Bp 6884 - 6905	XhoI site + bp 900-918 of CMVpromoter from gWIZ blank vector
Bp 6906 - 7860	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2)
Bp 7861 - 7866	Synthetic DNA added during vector construction (SalI site used for ligation)
Bp 7867 - 7929	Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7873) (from GenBank Accession # X02009)
Bp 7930 - 8427	Human Interferon alpha-α2b (IFN-α2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted
Bp 8428 - 8433	Synthetic DNA added during vector construction (BamHI site used for ligation)

Bp 8434 - 9349 Chicken Ovalbumin PolyA (taken from GenBank Accession # J00895; bp 8260-9176)

Bp 9350 – 15199 pTn10-Gen/Mar BV (Bp 5399-11248)

SEQ ID NO:21 Vector 262 pTn10-Gen/Mar BV (CMV.Ovalp vs. 1/n3xf/hIFNA/OvpyA)

Bp 1 - 5381 pTn10-Gen/Mar BV (bp 1-5381)

Bp 5382 - 6221 Chicken Ovalbumin Promoter (bp 1090-1929)

Bp 6222 - 6227 Synthetic DNA added during vector construction (EcoRI site used for ligation)

Bp 6228 - 6882 CMV enhancer/promoter (bp 245-899 of gWIZ blank vector)

Bp 6883 - 6904 XhoI site + bp 900-918 of CMVpromoter from gWIZ blank vector

Bp 6905 - 7859 CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2)

Bp 7860 - 7865 Synthetic DNA added during vector construction (SalI site used for ligation)

Bp 7866 - 7928 Chicken Conalbumin Signal Sequence + Kozak sequence (7866-7872) (from GenBank Accession # X02009)

Bp 7929 - 7934 Synthetic DNA added during vector construction (BsrFI site used for ligation)

Bp 7935 - 7985 3x flag

Bp 7986 - 8000 Enterokinase Cleavage Site

Bp 8001 - 8498 Human Interferon alpha-α 2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted

Bp 8499 - 8504 Synthetic DNA added during vector construction (BamHI site used from ligation)

Bp 8505 - 9420 Chicken Ovalbumin PolyA (taken from GenBank Accession # J00895, bp 8260-9176)

Bp 9421 – 15270 pTn10-Gen/Mar BV (bp 5399-11248)

SEQ ID NO:22 Vector #248-5021

pTn10 - Puro/Mar flanked BV (CMV/Ovalp vs. 1/CMViA'/Conss/hIFNA/SynpyA)

Bp 1 - 5381 pTn10 Puro/Mar flanked backbone vector (bp 1-5381)

Bp 5382 - 6228 Chicken Ovalbumin Promoter (bp 1090-1929), including synthetic DNA added during vector construction on 3' end

Bp 6229 - 6905	CMV enhancer/promoter, bp 245-899 of gWIZ blank vector CTC, bp 900-918 of CMVpromoter from gWIZ blank vector
Bp 6906 - 7866	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including synthetic DNA added during vector construction on 3' end
Bp 7867 - 7929	Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7873) (from GenBank Accession # X02009)
Bp 7930 - 8433	Human Interferon alpha-2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted; including synthetic DNA added during vector construction on 3' end
Bp 8434 - 8797	Synthetic polyA; taken from gWIZ blank vector (bp 1921-2334)
Bp 8798 - 14217	pTn10 Puro/Mar flanked backbone vector (bp 5385-10804)

SEQ ID NO:23 ID# 309 – HPvs1/ CMViA/ native hIFNα 2βss/ hIFNα 2β / OPA in pTN-10 PURO-MAR Flanked BV

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)
Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA

Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
Bp 5382 - 6223	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
Bp 6224 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)with 5' EcoRI RE site
Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 7861 - 7938	Native hINFα 2β Kozak (7867-7872) + Signal Peptide (Genbank Accession #J00207 bp 508-579) with 5' SalI RE site
Bp 7939 - 8436	Mature Interferon alpha 2 beta Gene (GenBank Accession #J00207 bp 580-1077)
Bp 8437 - 9358	Chicken Ovalbumin polyA from gDNA (GenBank Accession #J00895 bp 8260-9175)with 5' AgeI RE site
Bp 9359 – 9405	MCS extension from pTN-MCS, PacI thru BsiWI
Bp 9406 – 9718	HSV-TK polyA from pS65TC1 bp 3873-3561
BP 9719 - 10349	Puromycin resistance gene from pMOD PURO (invivoGen)
Bp 10350 - 10741	SV40 promoter from pS65TC1, bp 2232-2617 with 5' MluI RE site
Bp 10742 - 12446	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
Bp 12447 – 12516	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 12517 - 12559	Lambda DNA from pNK2859
Bp 12560 - 14764	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:24 Vector 310-5021

Puro/Mar (CMV.Ovalp vs1/Conss(-AA)/3xFlag/hIFN-α 2b(N-Gly)/OvpyA)

Bp 1 – 5381	Puro/Mar Backbone (bp 1-5381)
Bp 5382 - 6235	Chicken Ovalbumin Promoter (bp 1090-1929), including EcoRI site used for ligation on 3' end

Bp 6236 - 6912	CMV enhancer/promoter (bp 245-899 of gWIZ blank vector), CTC, bp 900-918 of CMVpromoter from gWIZ blank vector
Bp 6913 - 7873	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including SalI used for ligation on 3' end
Bp 7874 - 7933	Chicken Conalbumin Signal Sequence + Kozak sequence (7874-7879) (from GenBank Accession # X02009)
Bp 7934 - 7984	3xFlag
Bp 7985 - 7999	Enterokinase cleavage site
Bp 8000 – 8503	Human Interferon alpha-2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted; changed bp 790 from G to A to encode an N-glycosylation site, including synthetic DNA added during vector construction (BamHI site used for ligation) on 3' end
Bp 8504 - 9419	Chicken Ovalbumin PolyA site, taken from GenBank Accession # J00895 (bp 8260-9176)
Bp 9420 – 14825	Puro/Mar Backbone (bp 5399-10804)

SEQ ID NO:25 Vector 5021-311

Puro/Mar BV (CMV.Ovalp vs.1/Conss(-AA)/Mat.hIFNA(N-Gly)/OvpyA)

Bp 1 - 5381	pTN-10 Puro/Mar FBV (bp 1-5381)
Bp 5382 - 6228	Chicken Ovalbumin Promoter (bp 1090-1929), including EcoRI site used for ligation on 3' end
Bp 6229 - 6905	CMV enhancer/promoter (bp 245-899 of gWIZ blank vector), CTC, bp 900-918 of CMVpromoter from gWIZ blank vector
Bp 6906 - 7866	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including SalI site used for ligation on 3' end
Bp 7867 - 7926	Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7872) (from GenBank Accession # X02009)
Bp 7927 - 8430	Human Interferon alpha-α 2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted; changed bp 790 from G to A to encode N-glycosylation site, including synthetic DNA added during vector construction (BamHI site used for ligation) on 3' end

Bp 8431 - 9346	Chicken Ovalbumin PolyA site, taken from GenBank Accession # J00895 (bp 8260-9176)
Bp 9347 – 14752	Puro/Mar Backbone (bp 5399-10804)

SEQ ID NO:26 Vector #313 – HPvs1/ CMViA/ CAss + kozak/ Interferon-β 1a/ OPA in pTN-10 PURO-MAR Flanked BV

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)
Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
Bp 5382 - 6223	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
BP 6224 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)with 5' EcoRI RE site
Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)

BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ,Gene Therapy Systems)bp 1866-1873)
Bp 7861 - 7929	Kozak (7867-7872) + Conalbumin Signal Peptide (Genbank NM_205304 bp 74-133) with 5'SalI RE site
Bp 7930 - 8436	Interferon β 1a-codon optimized (GenBank NM_002176 bp 139-639)
Bp 8437 - 9352	Chicken Ovalbumin polyA from gDNA (GenBank #J00895 bp 8260-9175) with 5'AgeI RE site
Bp 9353 – 9399	MCS extension from pTN-MCS, PacI thru BsiWI
Bp 9400 – 9712	HSV-TK polyA from pS65TC1 bp 3873-3561
BP 9713 - 10343	Puromycin resistance gene from pMOD PURO (InvivoGen)
Bp 10344 - 10735	SV40 promoter from pS65TC1, bp 2617-2232 with 5' MluI RE site
Bp 10736 - 12440	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
Bp 12441 - 12510	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 12511 - 12553	Lambda DNA from pNK2859
Bp 12554 - 14758	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:27 Vector 286 Puro/Mar (CMV.Ovalp vs1/3xf/E.O.hIFNA2a/OvpyA)

Bp 1 - 5381	pTN-10 PURO MAR BV (bp 1-5381)
Bp 5382 - 6228	Chicken Ovalbumin Promoter (bp 1090-1929), including EcoRI site used for ligation) on 3' end
Bp 6229 - 6905	CMV enhancer/promoter (bp 245-899 of gWIZ blank vector), CTC, bp 900-918 of CMV promoter from gWIZ blank vector
Bp 6906 - 7866	CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including synthetic DNA added during vector construction (SalI cut site used for ligation) on 3' end
Bp 7867 - 7935	Chicken Conalbumin Signal Sequence + Kozak sequence (7878-7883) (from GenBank Accession # X02009), including BsrFI site used for ligation on 3' end
Bp 7936 - 7986	3x Flag
Bp 7987 - 8001	Enterokinase Cleavage Site

Bp 8002 - 8505 Human Interferon alpha-2a (IFN-α 2a) gene, Codon Context Optimized; corresponds to GenBank Accession # J00207 (bp 580-1077); Start codon omitted, site directed mutagenesis was done to change Arginine to lysine (bp 647, 648 changed from GA to AG), including synthetic DNA added during vector construction (BamHI site used for ligation) on 3' end

Bp 8506 - 9421 Chicken Ovalbumin PolyA (taken from GenBank Accession # J00895, bp 8260-9176)

Bp 9422 – 14827 pTN-10 PURO MAR BV (bp 5399-10804)

SEQ ID NO:28 Vector #295 Puro/Mar BV(CMV.Ovalp vs.1/Mat.hIFNA2b/OvpyA)

Bp 1 - 5381 pTN-10 PURO MAR BV (bp 1-5381)

Bp 5382 - 6228 Chicken Ovalbumin Promoter (bp 1090-1929), including EcoRI site used for ligation on 3' end

Bp 6229 - 6905 CMV enhancer/promoter (bp 245-899 of gWIZ blank vector), CTC, bp 900-918 of CMVpromoter from gWIZ blank vector

Bp 6906 - 7866 CMV intron A' (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including SalI site used for ligation on 3' end

Bp 7867 - 7929 Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7872) (from GenBank Accession # X02009)

Bp 7930 - 8433 Human Interferon alpha-2b (IFN-α 2b) gene, taken from GenBank Accession # J00207 (bp 580-1077); Start codon omitted, including synthetic DNA added during vector construction (BamHI site used for ligation) on 3' end

Bp 8434 - 9349 Chicken Ovalbumin PolyA (taken from GenBank Accession # J00895, bp 8260-9176)

Bp 9350 – 14755 pTN-10 PURO MAR BV (bp 5399-10804)

In one embodiment, the present application provides a novel sequence comprising a promoter, a gene of interest, and a poly A sequence. Each of these novel sequences may be identified from the annotations for each expression vector shown above, and also as sequences within the sequence listing for each expression vector. The specific bases of these novel sequences are provided in Table 3 below for each expression vector SEQ ID NOs:17 to 28.

Table 3

IFN Vectors		
SEQ ID NO	Begin	End
17	4929	7627
18	5382	8902
19	5382	8523
20	5382	9349
21	5382	9420
22	5382	8797
23	5382	9358
24	5382	9419
25	5382	9346
26	5382	9352
27	5382	9421
28	5382	9349

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various embodiments, modifications and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the invention.

EXAMPLE 1

Preparation of Vectors for Expression of Interferon

Construction of Vector #188 (SEQ ID NO:17)

The pTopo vector containing an IFN-α 2b cassette driven by the CMV promoter was digested with restriction enzyme Asi SI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the interferon cassette into the MCS of p5012 (SEQ ID NO:4), the purified IFN-α 2b DNA and p5012 were digested with Asi SI, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical

transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until needed.

Construction of Vectors #206 (SEQ ID NO:18) and 207 (SEQ ID NO:19)

The pTopo vectors containing the IFN-α 2b cassettes driven by either the hybrid promoter version 1 (SEQ ID NO:14) or version 2 (SEQ ID NO:15) were digested with restriction enzyme Asi SI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the IFN-α 2b cassette into the MCS of p5021 (SEQ ID NO:8), the purified IFN-α 2b DNA and p5021 were digested with Asi SI, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250

ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

Construction of Vector #261 (SEQ ID NO:20)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the human interferon-α 2b (hIFNα 2b) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14), was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hIFN-α 2b cassette into the MCS of p5022 (SEQ ID NO:9), purified hIFN-α 2b DNA and p5022 were digested with AscI and PacI, purified as described above, and ligated using New England Biolab's Quick T4 DNA Ligase Kit (Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the hIFN-α 2b cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in

250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #262 (SEQ ID NO:21)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the human interferon-α 2b (hIFN-α 2b) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14), was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hIFN-α 2b cassette into the MCS of p5022 (SEQ ID NO:9), purified hIFN-α 2b DNA and p5022 were digested with AscI and PacI, purified as described above, and ligated using New England Biolab's Quick T4 DNA Ligase Kit (Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad. CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the hIFN-α 2b cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #248 (SEQ ID NO:22)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the human interferon-α 2b (hIFN-α 2b) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14), was digested with restriction enzymes AscI and AsiSI (Fermentas, Glen Burnie, MD) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hIFN-α 2b cassette into the MCS of p5021 (SEQ ID NO:8), purified hIFN-α 2b DNA and p5021 were digested with AscI and AsiSI, purified as described above, and ligated using New England Biolab's Quick T4 DNA Ligase Kit (Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the hIFN-α 2b cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #309 (SEQ ID NO:23)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the mature interferon alpha 2b (hIFN-α 2b) cassette driven by the hybrid promoter version 1 (SEQ ID #14) was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using a Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the mature hIFN-α 2b cassette into the MCS of p5021 (SEQ ID NO:8), purified mature hIFN-α 2b DNA and p5021 were digested with AscI and PacI,

purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the mature hIFN-α 2b cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500μL of endotoxin free water and stored at -20°C until needed.

Construction of Vectors #310 (SEQ ID NO:24) and #311 (SEQ ID NO:25)

A human interferon-α 2b cassette was modified to encode an N-glycosylation site at amino acid 71 of the protein (SEQ ID NO:29). This was the result of a single substitution of a guanine to an adenine residue at bp 790 of the nucleotide sequence (SEQ ID NO:30), resulting in a single amino acid substitution of aspartic acid to asparagine at amino acid 71 of the protein (SEQ ID NO:29). The resulting cassette was named human interferon-α 2b N-glycosylated (hIFN-α 2b (N-Gly)). Western blot analysis with protein produced by this vector supports the concept that the encoded protein does in fact become N-glycosylated, as that protein migrated more slowly in the gel than protein expressed from a vector with an unmodified hIFN-α 2b cassette (data not shown). Similarly, when the hIFN-α 2b (N-Gly) protein was digested with PNGase F (which cleaves N-glycosylation sites) prior to electrophoresis, the band for the digested protein shifted to a lower molecular weight.

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the hIFN-α 2b (N-Gly) cassette driven by the hybrid promoter version 1 (SEQ ID #14), was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hIFN-α 2b (N-Gly) cassette into the MCS of p5021 (SEQ ID NO:8), purified hIFN-α 2b (N-Gly) DNA and p5021 were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the hIFN-α 2b (N-Gly) cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500μL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #313 (SEQ ID NO:26)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the interferon-beta 1a (hINF-β 1a) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14) was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hINFβ-1a cassette into the MCS of p5021 (SEQ ID NO:8), purified hINF-β 1a DNA and p5021 were digested with AscI and PacI, purified as described above, and

ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the hINF-β 1a cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500μL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #286 (SEQ ID NO:27)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the codon optimized human interferon-α 2a (C.O. hIFN-α 2a) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14), was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the C.O. hIFN-α 2a cassette into the MCS of p5021 (SEQ ID NO:8), purified C.O. hIFN-α 2a DNA and p5021 were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 μg/ml ampicillin

(LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the C.O. hIFN-α 2a cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500μL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #295 (SEQ ID NO:28)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the human interferon-α 2b (hIFN-α 2b) cassette driven by the hybrid promoter version 1 (SEQ ID NO:14), was digested with restriction enzymes AscI and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using Zymo Research's DNA Clean and Concentrator kit (Orange, CA). To insert the hIFN-α 2b cassette into the MCS of p5021 (SEQ ID NO:8), purified hIFN-α 2b DNA and p5021 were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.25 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB (Luria-Bertani) agar plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-

Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained the hIFN-α 2b cassette, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using Qiagen's EndoFree Plasmid Maxi-Prep kit (Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500μL of endotoxin free water and stored at -20°C until needed.

Vector Maps and Sequences

Schematics of some of the disclosed vectors (#188 (SEQ ID NO:17), #206 (SEQ ID NO:18), and #207 (SEQ ID NO:19)) are shown in Figures 2A, 2B, and 2C respectively. The sequences of these vectors, as well as the sequences of the other disclosed interferon expression vectors, are shown below in the Appendix. A schematic of the resulting mRNA transcript for vectors #188, #206, and #207 is shown in Figure 2D. These vectors were used to analyze expression of and bioactivity of IFN-α 2b as shown in the following examples.

EXAMPLE 2

In Vitro Expression of hIFN-α 2b in LMH2A Cells

These experiments were performed to verify that the IFN expression vectors (#188 (SEQ ID NO:17), #206 (SEQ ID NO:18), and #207 (SEQ ID NO:19)) produced hIFN-α 2b protein and to determine whether the hIFN-α 2b product was toxic to the transfected cells.

The graph in Figure 3 shows the ELISA readings for the media samples from one of these experiments. T1 & T2 are duplicate flasks. Control flasks also were run, but the readings were too low to detect at these dilution levels (data not shown). The M1 samples were estimated to contain on the order of approximately 5 μg/ml interferon. The #206 vector and #207 vector efficiently expressed 3xFlag hIFN-α 2b. The M1 samples were estimated to contain on the order of approximately 19 or 15 μg/ml interferon, respectively (data not shown).

Western blots also were performed, and a protein of the expected size was detected, both with 3xFlag antibody and antibody directed against the interferon portion of the molecule (data not shown). In those experiments, media from two different flasks containing LMH2A cells transfected with the hIFN-α 2b expression vector was analyzed at two to four different timepoints after transfection. After running the proteins on an SDS-PAGE gel and immunoblotting the gel,

the immunoblot was incubated with either an anti-3xFlag antibody or an anti-IFN antibody. These data demonstrated the induction of expression of hIFN-α 2b in LMH2A cells that were transfected with the hIFN-α 2b expression vector, but not in un-transfected control cells. In those experiments, the 3xFlag hIFN-α 2b runs slower in the gel than the recombinant hIFN-α 2b standard due, at least in part, to the increased molecular weight added by the 3xFlag epitope.

There was no indication that the product produced was toxic in any way to the cells. The cells remained alive, healthy, and demonstrated typical morphology throughout the experiment.

EXAMPLE 3

Purification of IFN-α 2b from Culture Media

As shown in Figure 2D, the IFN-α 2b transcript was produced with a signal sequence and 3xFlag moiety on the N-terminal portion of the sequence. The resulting fusion protein was produced in the transfected cells, and then the signal sequence was cleaved in the endoplasmic reticulum prior to the secretion of the 3xFlag-IFN-α 2b into the culture media. The IFN-α 2b protein was purified from the culture media by means of the 3xFlag moiety. In order to produce the mature IFN-α 2b protein from purified recombinant 3xFlag-IFN-α 2b protein, it was necessary to remove the amino-terminal 3xFlag epitope by enterokinase digestion. Recombinant enterokinase (Novagen) was added to the purified 3xFlag-IFN-α 2b protein at a ratio of 1.0 Unit of enterokinase to 50 μg of 3xFlag-IFN-α 2b. The reaction was incubated at room temperature for 16 hours with gentle agitation.

Following enterokinase digestion, the resulting proteins and fragments thereof were run on an SDS PAGE gel (data not shown). Removal of the 3xFlag epitope was evidenced by a band shift on the Coomassie stained SDS-PAGE gel in which the enterokinase digested 3xFlag-IFN migrated at a lower molecular weight relative to the undigested 3xFlag-IFN. The gel shows that the banding pattern was similar in the enterokinase digests as with the control samples (in which no enterokinase was added) with the exception that the pattern shifted down to a lower molecular weight. This shift suggests that the N-terminal 3xFlag eptiope was in fact removed. Additionally, Western blot analysis indicated that the 3xFlag epitope was no longer present on the enterokinase digested 3xFlag-IFN when the blot was probed against anti-Flag immunoglobulins (data not shown). Moreover, no "alternative" cleavage sites were evident (*i.e.*, due to potential "overdigestion").

The remaining IFN expression vectors also have been assayed for their ability to produce mature IFN-α 2a, IFN-α 2b, or IFN-β 1a either initially as the mature protein or initially as a

3xFlag tagged IFN-α 2a, IFN-α 2b, or IFN-β 1a, followed by purification as discussed in this example. Typical results for the expression vectors are shown in Table 4.

Table 4

Vector number (SEQ ID NO)	Cell type	IFN	Amount of protein
188 (SEQ ID NO:17)	LMH2A	3xFlag IFN-α 2b	1.3 μg/ml
206 (SEQ ID NO:18)	LMH	3xFlag IFN-α 2b	2.6 μg/ml
206 (SEQ ID NO:18)	LMH2A	3xFlag IFN-α 2b	1.9 μg/ml
248 (SEQ ID NO:22)	LMH	IFN-α 2b	5.0 μg/ml
248 (SEQ ID NO:22)	LMH2A	IFN-α 2b	2.9 μg/ml
261 (SEQ ID NO:20)	LMH	IFN-α 2b	12.9 μg/ml
262 (SEQ ID NO:21)	LMH	3xFlag IFN-α 2b	12.9 μg/ml
295 (SEQ ID NO:28)	LMH	IFN-α 2b	10 μg/ml
295 (SEQ ID NO:28)	LMH2A	IFN-α 2b	4.5 μg/ml
309 (SEQ ID NO:23)	LMH2A	IFN-α 2b	1.6 μg/ml
310 (SEQ ID NO:24)	LMH2A	3xFlag IFN-α 2b	1.75 μg/ml
311 (SEQ ID NO:25)	LMH2A	IFN-α 2b	1.2 μg/ml

These data demonstrate the efficient production and purification of the mature or 3xFlag IFN-α 2b protein using the presently disclosed compositions.

EXAMPLE 4

In Vitro Assay of hIFN-α 2b Bioactivity

These experiments were performed to verify that the IFN-α 2b produced by one of the vectors (# 188 (SEQ ID NO:17)) in the transfected cells was a bioactive IFN-α 2b. Table 5 shows the results of luminescence assays.

Table 5

Sample	Luminescence
200 IU/ml standard	1597
6.25 IU/ml standard	242
Pur IFN diluted 10^2	1809
Pur IFN diluted 10^4	1116
Pur IFN diluted 10^5	295
Pur 3xFlag-IFN diluted 10^2	1611
Pur 3xFlag-IFN diluted 10^5	1119
Pur 3xFlag-IFN diluted 10^6	184
Negative Control	41

Specific activity standards were provided by the iLite™ Human Interferon Alpha Kit (Interferon Source, Piscataway, NJ) and were prepared according to the manufacturer's instructions. The iLite™ kit allows for a quantitative determination of human interferon alpha bioactivity using luciferase generated bioluminescence. The kit is suitable for detection of the activity of other human interferons, and not just hIFN-α 2b.

The test samples were prepared according to the manufacturer's conditions. In this table, "Pur IFN" refers to a sample in which the 3xFlag IFN-α 2b produced was subjected to enterokinase digestion prior to the bioassay. "Pur 3xFlag-IFN" refers to a sample in which the 3xFlag IFN-α 2b produced was not subjected to enterokinase digestion prior to the bioassay.

Both the mature IFN-α 2b and 3xFlag IFN-α 2b generated significant bioluminescence when compared to the standards and negative control, as shown in Table 5. As may be expected, the 3xFlag IFN-α 2b sample appeared to have greater activity than the enterokinase digested sample, when comparing greater dilutions of the mature and 3xFlag IFN-α 2b test samples. Based on a comparison of the IFN-α 2b results with the standards and negative control sample, these results demonstrate that the IFN-α 2b produced by this expression vector was bioactive.

EXAMPLE 5

In Vitro Expression of hIFN-α 2b in LMH Cells

This experiment tests a new vector for its efficiency of expression of mature IFN-α 2b in LMH2A cells. The CMV.ovalp vs1 (SEQ ID NO:14) is the promoter driving the expression of native interferon in vector #248 (SEQ ID NO:22). For comparison purposes, vector #206 (SEQ

ID NO:18), which comprises the same promoter driving expression of a gene encoding the 3xFlag-Interferon was used. Triplicate samples of LMH2A cells were transfected with either vector #248 or vector #206.

Transfection was carried out by the standard Fugene 6 protocol using 2 μg DNA/flask and Fugene 6:DNA at 6:1. The cultures were grown on Waymouth's + 10% FCS with no antibiotic for 48 hours, and then fed with Waymouth's + 5% FCS + G418 antibiotic when samples were taken. Samples were taken at 2 days post-transfection (M1), 6 days post-transfection (M2), and 9 days post-transfection (M3). The data is presented in a single graph shown in Figure 4; however, two separate standard curves were used in the sandwich ELISA format for the native and fusion protein. The standard curve used for the quantification of native protein was commercial recombinant human interferon (rhIFN) at known concentrations, while the standard curve for the quantification of the fusion protein was the inventors' 3xFlag-interferon at known concentrations.

The expression of the native interferon from vector #248 (SEQ ID NO:22) in LMH2A cells appears to be extremely efficient, achieving more than double the amount of expression of the fusion protein from vector #206 (SEQ ID NO:18).

EXAMPLE 6

Efficiency of Transfection of LMH and LMH2A Cells

To determine whether certain cell types and certain vectors were capable of increased expression of interferon, the following experiment was conducted. As in Example 5, vector #206 (SEQ ID NO:18) and vector #248 (SEQ ID NO:22) were used to transfect either LMH or LMH2A cells.

Each vector DNA dilution was quantified by GeneQuant (AMB) and normalized in the transfection to deliver precisely 2 μg DNA/T25 flask. The cells were transformed using the standard Fugene 6 protocol using 2 μg DNA/flask and Fugene 6:DNA at 6:1. Complex formation was done in Waymouth's (no additives), and the transfection was done in Waymouth's +10% FBS +HEPES (no antibiotics). After 48 hours, the cultures were grown on Waymouth's + 5% FCS +HEPES (+/- G418 antibiotic).

Following normalized transfection of a standard number of cells, Sandwich ELISA (for 3xFlag IFN-α 2b) and Inhibition ELISA experiments (for mature IFN-α 2b) were conducted, and the results are shown in Figure 5. (Alternatively, Sandwich ELISA may be used with mature IFN-α 2b as well, or in the place of the Inhibition ELISA experiments.) Samples were taken at 3 days post-transfection, 7 days post-transfection, and 10 days post-transfection. The data presented

in Figure 5 are reported in micrograms/ml. As shown in Figure 5, both the LMH cells and the LMH2A cells produced IFN-α 2b. The inhibition ELISA assay used a commercial IFN-α 2b standard in the standard curve, and the sandwich ELISA standard curve relied on the inventors' purified 3xFlag-hIFN-α 2b for quantification.

5

EXAMPLE 7

Perfusion of LMH2A Cells in AutoVaxID

The AutoVaxID cultureware (Biovest, Worcester, MA) was installed, and the Fill-Flush procedure was performed following the procedures in the AutoVaxID Operations Manual. The 10 following day, the pre-inoculation procedure and the pH calibration were done. The cultureware was seeded with 10^9 LMH2A cells transfected with an expression vector IFN-α 2b (#261)(SEQ ID NO:20). The cells were propagated in Lonza UltraCULTURE media supplemented with cholesterol (Sigma, 50 μg/ml) in 20 gelatin-coated T150 cell culture flasks, and were dissociated with Accutase (Sigma). They were counted, gently pelleted (600xG for 6 minutes), and 15 resuspended in 50 mls of growth media (Lonza UltraCULTURE containing GlutaMax (Invitrogen) and SyntheChol (1:500), Soy Hydrolysate (1:50), and Fatty Acid Supplement (1:500) (all from Sigma). This was the same media which was included in the "Factor" bags for the AutoVaxID, used for the EC (extra-capillary) media. A 10 L bag of Lonza UltraCULTURE media (with GlutaMax) was used initially for the IC (intra-capillary) media. This was designed to 20 give the cells a richer media for the first 7-10 days, to allow them to become established quickly in the hollow fiber system. After this bag was exhausted, the IC media was switched to DMEM/F12 (also including GlutaMax), also purchased from Lonza. This media was purchased in 50 L drums, and was removed from the cold room and allowed to warm to room temperature before being connected to the system. The AutoVaxID system was placed under Lactate Control, 25 and pump rates were modified and daily tasks performed, as specified by the AutoVaxID Operating Procedures Manual, provided by the manufacturer (Biovest).

Six days later, cells were seen growing on the hollow fibers in the bioreactor. Up until this time, there was ample evidence that the cells were growing and metabolizing in the system; the Lactate Controller was increasing the media pump rate regularly in order to keep the lactate levels 30 below the setpoint, and the pH Controller was continually decreasing the percentage of CO_2 in the gas mix, indicating that the cells were producing increasing amounts of acidic metabolic products. After the IC media was changed from the Lonza UltraCULTURE media to the DMEM/F12, however, the metabolic rate of the cells may slow dramatically, to the point where the Lactate Controller slows the media pumps all the way to baseline levels , and the lactate

levels may still drop. Samples were taken for protein analysis 4 days later. Samples were taken from the EC (showing current production) from the Harvest Bag (showing accumulated production) and from the IC (showing any protein which crossed the membrane and was lost in the wasted media). Four days later, there were both visual and metabolic evidence that the cells were growing, so cycling was initiated. For the next week, regular sampling was continued, and cells appeared to grow and metabolize normally. The run was allowed to continue for a couple weeks, although cycling times became greatly extended. Final samples were taken, and the run was ended. All samples were analyzed for proteins to determine if the cells are capable of producing significant amounts of protein in this system. In one such experiment with the AutoVax ID system, cells cultured in this way were taken twice a week over a 70 day period. Approximately 1.9 gram of IFN α2b were produced in approximately 1.5 L.

EXAMPLE 8

Production of transgenic chicken and quail that successfully pass the IFN

Separate *in vivo* experiments in chicken and quail are conducted to demonstrate successful passage of the transgene encoding a hIFN through two generations. Briefly, germ line cells of both chicken and quail are made transgenic following administration of one of the disclosed hIFN expression vectors (SEQ ID NOs:17-28) into the left cardiac ventricle, the source of the aorta which provides an artery leading to the ovary. These birds are mated with naïve males and the resulting eggs hatched. The resulting chicks (G1 birds) contain the transgene encoding hIFN, as is demonstrated when their blood cells are positive for the transgene encoding hIFN. These transgenic progeny (G1 birds) are subsequently bred, and their progeny (G2 birds) are positive for the transgene encoding hIFN.

Transgenic G1 and G2 quail are generated by injecting females in the left cardiac ventricle. The experiment uses five seven-week old quail hens. The hens are each injected into the left ventricle, allowed to recover, and then mated with naïve males. Isofluorane is used to lightly anesthetize the birds during the injection procedure. Eggs are collected daily for six days and set to hatch on the seventh day. At about 2 weeks of age, the chicks are bled and DNA harvested as described in a kit protocol from Qiagen for isolating genomic DNA from blood and tissue. PCR is conducted using primers specific to the gene of interest. Transgene-positive G1 animals are obtained. These transgene-positive G1 animals are raised to sexual maturity and bred. The G2 animals are screened at 2 weeks of age, and transgenic animals are identified in each experiment.

One of the hIFN expression vectors (SEQ ID NOs:17-28) is injected. In one embodiment, a total of 85 μg complexed with branched polyethyleneimine (BPEI) in a 300 μL total volume is used. G1 and G2 quail are positive for the hGH transgene following analysis of blood samples.

Transgenic G1 and G2 chickens are generated by injecting females in the left cardiac ventricle. This experiment is conducted in 20 week old chickens. One of the hIFN expression vectors (SEQ ID NOs:17-28) as described above for quail is injected. DNA (complexed to BPEI) is delivered to the birds at a rate of 1 mg/kg body (up to 3 ml total volume) weight by injection into the left cardiac ventricle. Isofluorane is used to lightly anesthetize the birds during the injection procedure. Once the birds recover from the anesthesia, they are placed in pens with mature, naïve males. All eggs are collected for 5 days and then incubated. In this experiment, the eggs are incubated for about 12 days, candled to check for viable embryos; any egg showing a viable embryo is cracked open and tissue samples (liver) taken from the embryo for PCR. The eggs are allowed to hatch, and a blood sample is taken at two days to test the animals for the presence of the transgene using PCR.

All patents, publications and abstracts cited above are incorporated herein by reference in their entirety. It should be understood that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the present invention as defined in the following claims.

CLAIMS

We claim:

1. A vector comprising:

a modified transposase gene operably linked to a first promoter, wherein the nucleotide sequence 3' to the first promoter comprises a modified Kozak sequence, and wherein a plurality of the first twenty codons of the transposase gene are modified from the wild-type sequence by changing the nucleotide at the third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon;

a multiple cloning site;

transposon insertion sequences recognized by a transposase encoded by the modified transposase gene, wherein the transposon insertion sequences flank the multiple cloning site; and,

one or more insulator elements located between the transposon insertion sequences and the multiple cloning site.

2. The vector of claim 1 comprising any one of SEQ ID NOs: 2 to 13.

3. The vector of claim 1, wherein the vector comprises any one of SEQ ID NOs: 10 to 13.

4. The vector of claim 1, further comprising a second promoter, wherein the second promoter is SEQ ID NO: 14 or SEQ ID NO: 15.

5. The vector of claim 4, further comprising a gene encoding for interferon inserted into the multiple cloning site.

6. The vector of claim 5, wherein the vector comprises any one of SEQ ID NOs: 17 to 28.

7. A promoter comprising chicken ovalbumin promoter regulatory elements in combination with a cytomegalovirus enhancer and a cytomegalovirus promoter.

8. The promoter of claim 7 comprising SEQ ID NO: 14.

9. A promoter comprising a steroid dependent response element, a cytomegalovirus enhancer, a chicken ovalbumin negative response element and a cytomegalovirus promoter.

10. The promoter of claim 9 comprising SEQ ID NO: 15.

11. A transposon-based vector comprising:

a modified transposase gene operably linked to a first promoter, wherein the nucleotide sequence 3' to the first promoter comprises a modified Kozak sequence, and wherein a plurality of the first twenty codons of the transposase gene are modified from the wild-type sequence by changing the nucleotide at the third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon;

one or more genes of interest encoding interferon operably-linked to one or more additional promoters, wherein the one or more genes of interest encoding interferon and their operably-linked promoters are flanked by transposon insertion sequences recognized by a transposase encoded by the modified transposase gene; and,

one or more insulator elements located between the transposon insertion sequences and the one or more genes of interest encoding interferon.

12. The vector of claim 11, wherein the vector comprises any one of SEQ ID NOs: 17 to 28.

13. A method of producing interferon comprising:

transfecting a cell with a vector comprising a modified gene encoding for a transposase, a promoter and a gene encoding for interferon;

culturing the transfected cell in culture medium;

permitting the cell to release interferon into the culture medium;

collecting the culture medium; and,

isolating the interferon.

14. The method of claim 13 wherein the vector comprises any one of SEQ ID NOs: 17 to 28.

15. The one or more insulator elements of any of the preceding claims comprising an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, or a matrix attachment region element.

16. The interferon of any of the preceding claims, wherein the interferon is human interferon.

17. An interferon protein comprising the sequence of SEQ ID NO:29.

18. A nucleotide sequence encoding for the interferon protein of Claim 17, wherein the nucleotide sequence comprises SEQ ID NO:30.

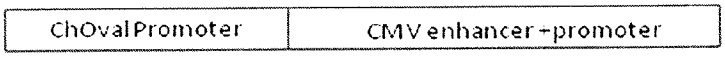
WO 2010/036979

Title: Novel Vectors for Production of Interferon
Filed: September 25, 2009
Inventors: Richard K. Cooper and William C. Fioretti
Attorney Docket No. 51687-382207

PCT/US2009/058498

FIG. 1

A.



B.

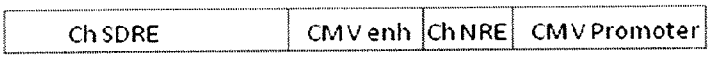
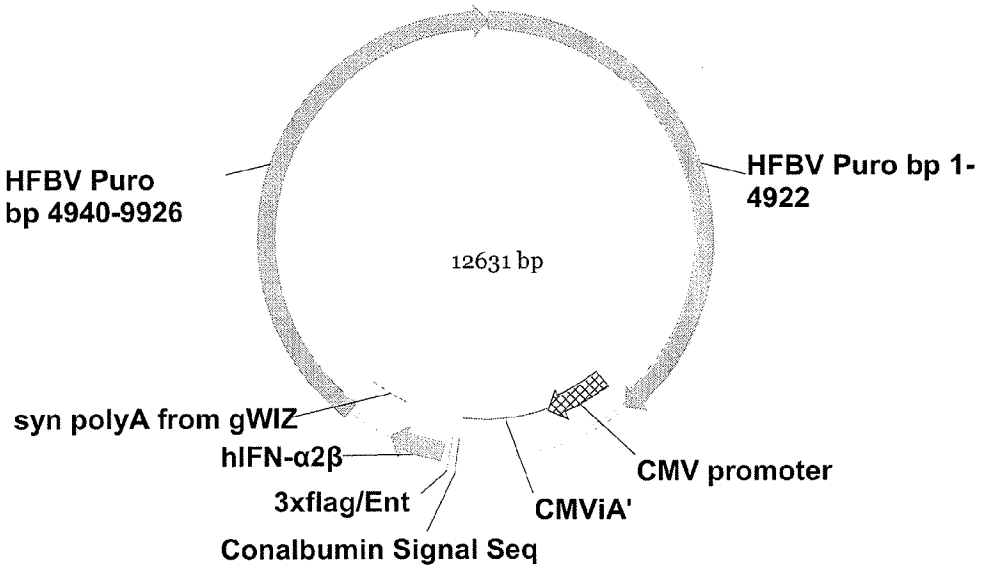
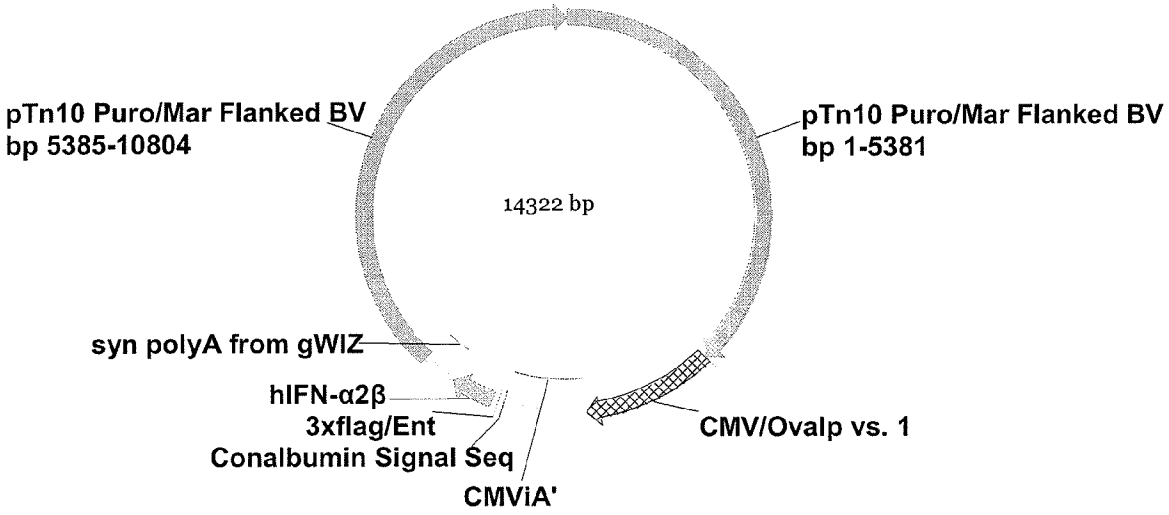


FIG. 2

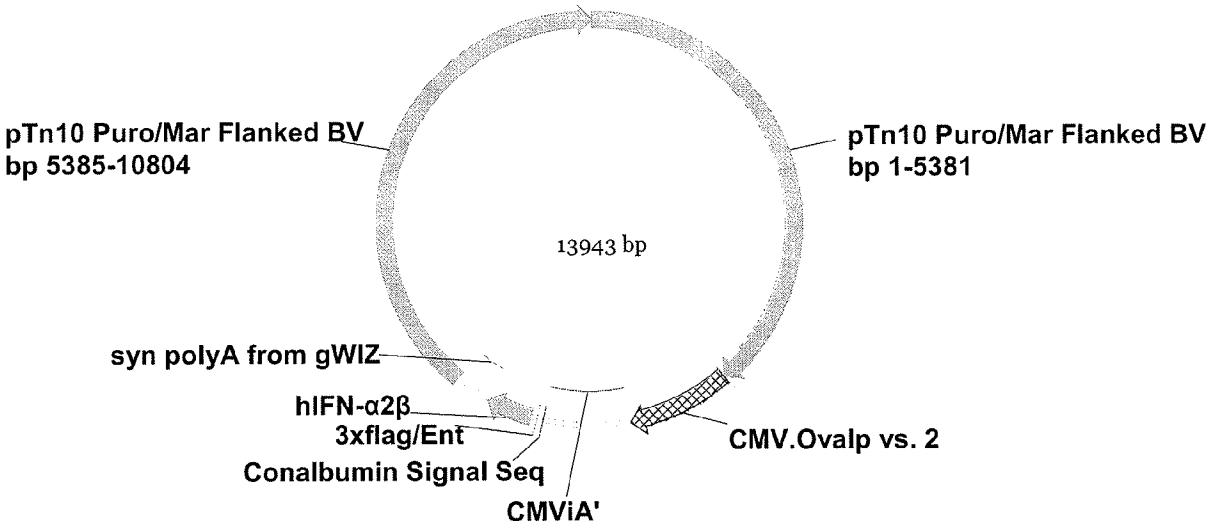
A.


HFBV Puro
bp 4940-9926
HFBV Puro bp 1-
4922
12631 bp
syn polyA from gWIZ
hIFN-α2β
3xflag/Ent
Conalbumin Signal Seq
CMV promoter
CMViA'

B.


pTn10 Puro/Mar Flanked BV
bp 5385-10804
pTn10 Puro/Mar Flanked BV
bp 1-5381
14322 bp
syn polyA from gWIZ
hIFN-α2β
3xflag/Ent
Conalbumin Signal Seq
CMViA'
CMV/Ovalp vs. 1

C.



D.

Signal Sequence	3xFlag	Ent	Interferon Alpha 2b	Poly A

FIG. 3

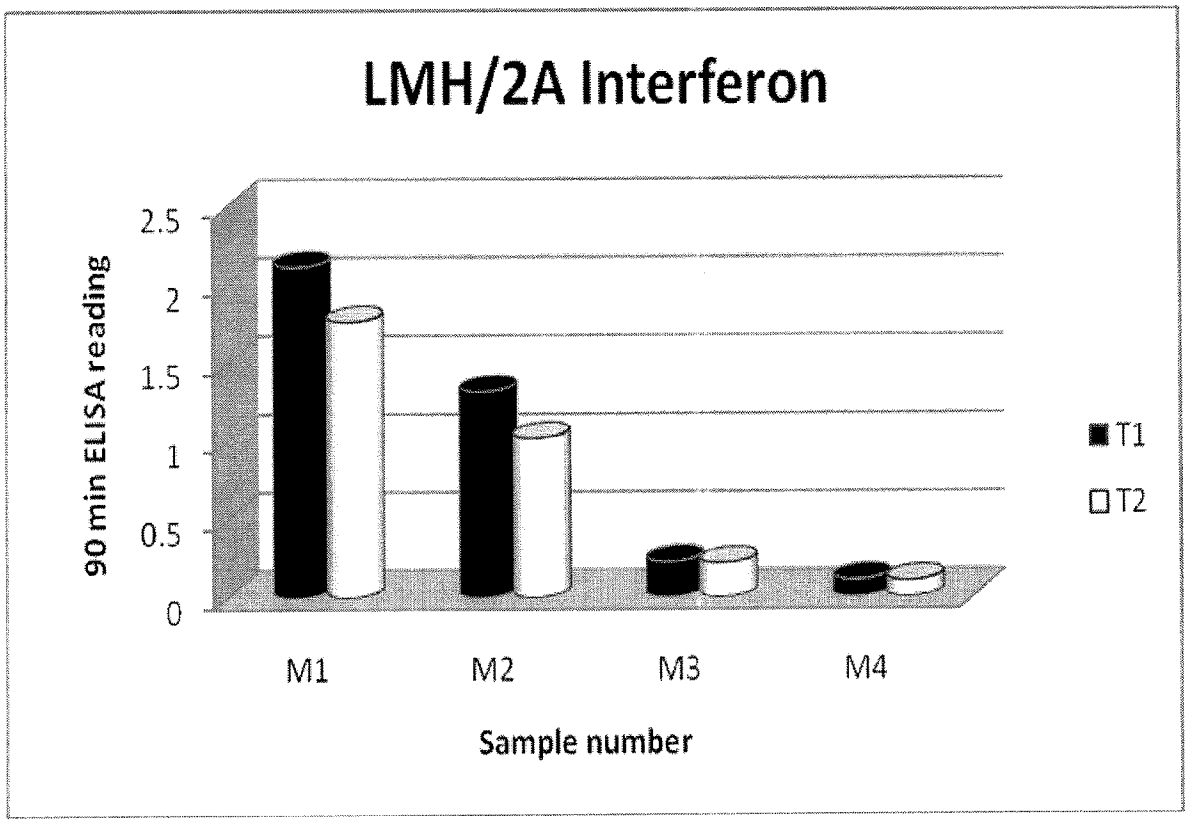
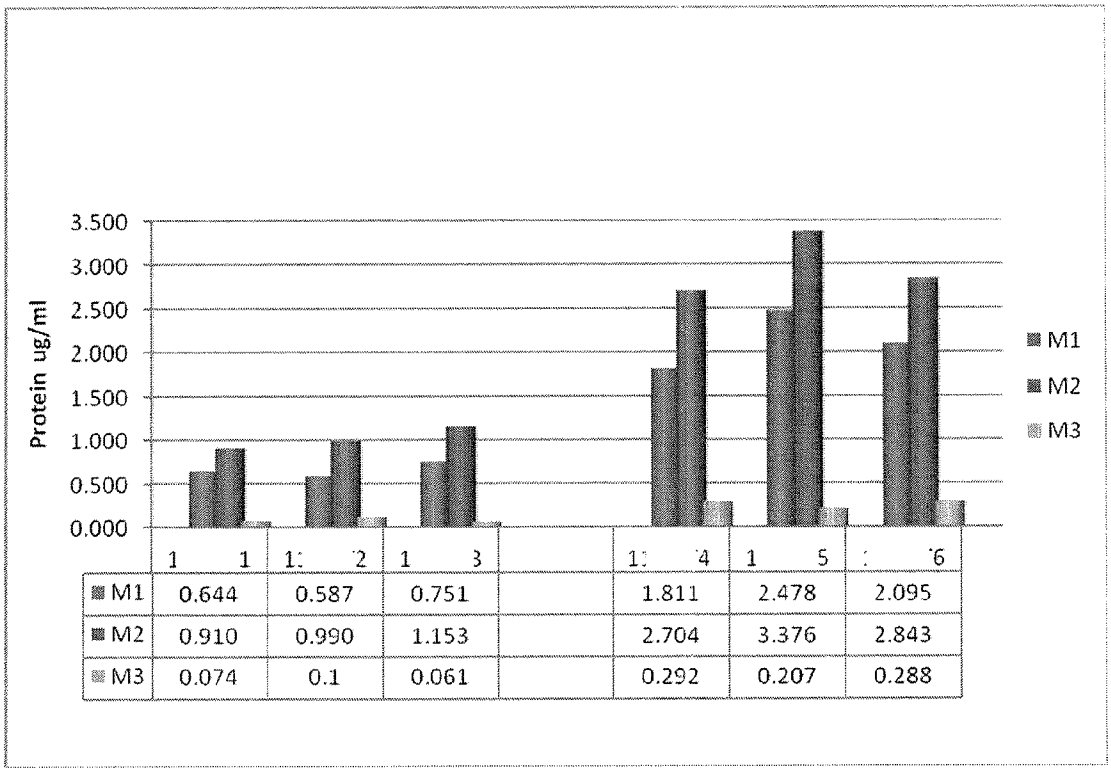
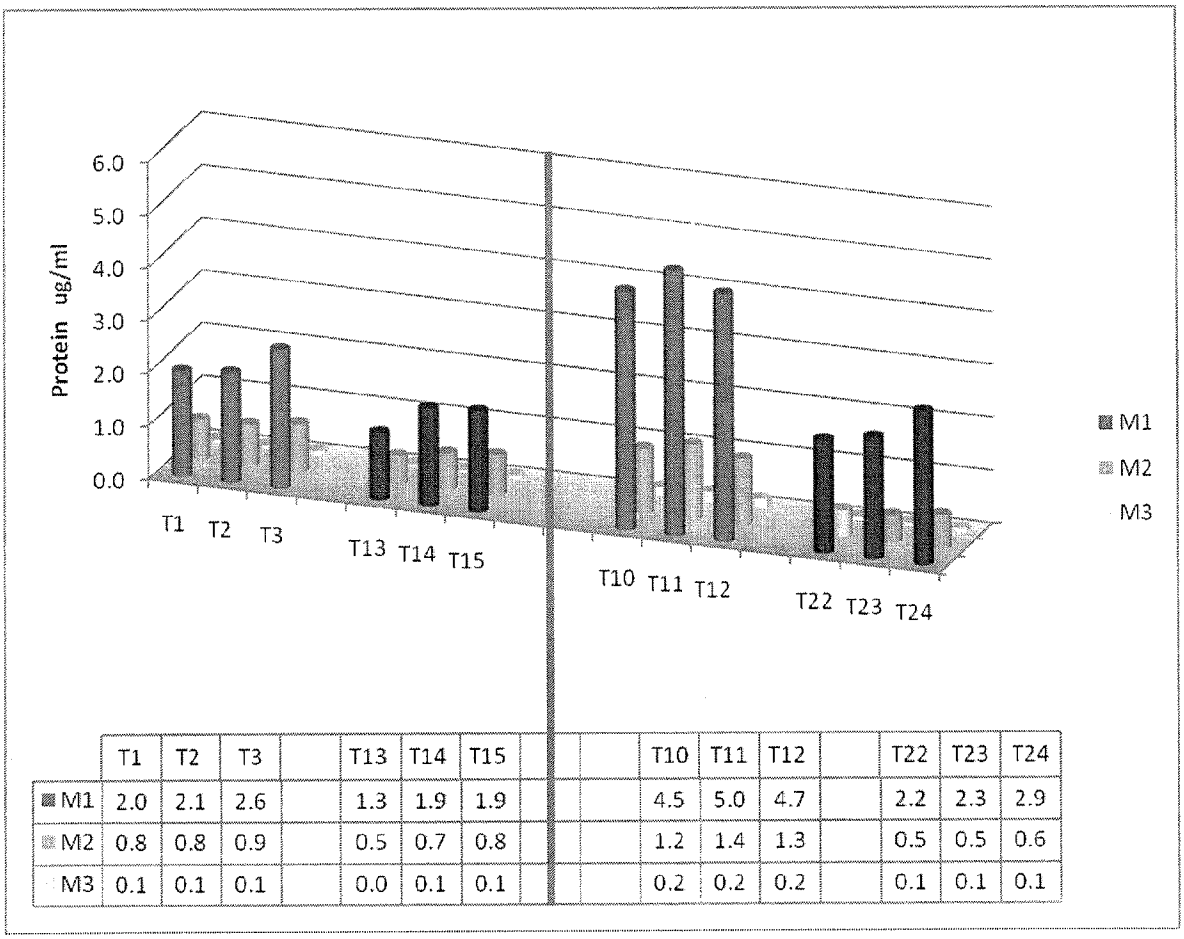

LMH/2A Interferon
90 min ELISA reading
2.5
2
1.5
1
0.5
0
M1
M2
M3
M4
Sample number
T1
T2

FIG. 4



	1 1	1 2	1 3		1 4	1 5	1 6
M1	0.644	0.587	0.751		1.811	2.478	2.095
M2	0.910	0.990	1.153		2.704	3.376	2.843
M3	0.074	0.1	0.061		0.292	0.207	0.288

FIG. 5



	T1	T2	T3		T13	T14	T15				T10	T11	T12		T22	T23	T24
M1	2.0	2.1	2.6		1.3	1.9	1.9				4.5	5.0	4.7		2.2	2.3	2.9
M2	0.8	0.8	0.9		0.5	0.7	0.8				1.2	1.4	1.3		0.5	0.5	0.6
M3	0.1	0.1	0.1		0.0	0.1	0.1				0.2	0.2	0.2		0.1	0.1	0.1